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SCLEROTINIA SPECIES CAUSING DECAY OF VEGETABLES UNDER TRANSIT AND MARKET CONDITIONS[1]

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INTRODUCTION

Sclerotinia sclerotiorum (Lib.) Massee (*Sclerotinia libertiana* Fckl.)[3] has long been recognized as an important pathogene in Europe. As early as 1886 DeBary (*2*)[4] made a comprehensive study of this fungus and showed that it was able to produce disease in a variety of plants. In America, apparently, no reference was made to this fungus until about 1890, when it was found associated with lettuce diseases of the type called "drop." It remained for R. E. Smith (*28*), however, to definitely establish, in 1900, the causal relationship of *S. libertiana* to lettuce "drop" and to clear up much of the misunderstanding regarding the life history of this fungus. Previous to this time several diseases of this type were fairly well known, but the causal organisms had not been established. This is shown by the articles of Humphrey (*17*), Selby (*26*), Stone and Smith (*30*), and Ramsey (*25*). Undoubtedly Sclerotinia, Pythium, Botrytis, and Rhizoctonia were individually and collectively responsible for most of the diseases described by these investigators.

PURPOSE OF STUDY AND SOURCE OF MATERIAL

At the present time it is found not only that *Sclerotinia libertiana* and related species are causing enormous damage in the field, but also that these fungi are the cause of some of the worst storage and transit troubles. It was with the purpose of becoming better acquainted with the latter type of decay and the causal organisms involved that the present work was undertaken.

In this study four main objects were kept in view: (1) To collect a large number of cultures from all available hosts and to make morphological and physiological studies, to determine, if possible,

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[2] The writer is greatly indebted to Dr. Geo. K. K. Link of the University of Chicago for valuable suggestions and for criticism of the manuscript. Thanks are also due Prof. H. H. Whetzel of Cornell University for suggestions on the studies of the microconidia and for the use of his laboratory during the month of July, 1920.

[3] According to the rules of nomenclature, the name *Sclerotinia sclerotiorum* (Lib.) Massee, is to be preferred, but since the name *S. libertiana* Fckl. has been used exclusively in American literature, it seems best to carry this name in the present paper in order to avoid confusion (*31*).

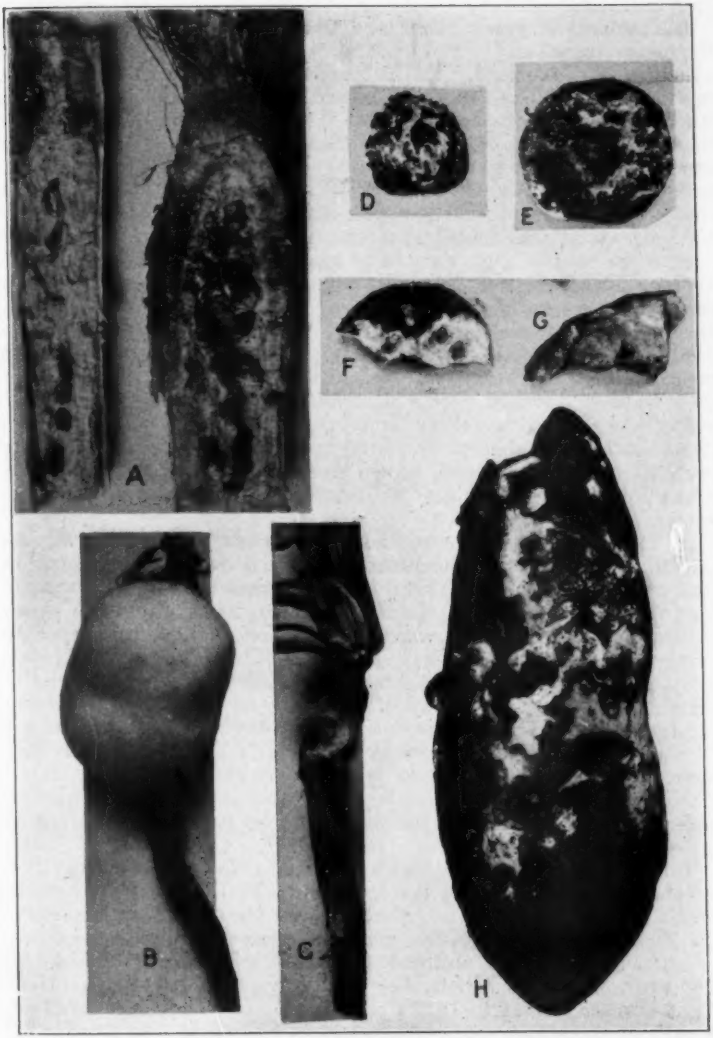
[4] Reference is made by number (italic) to "Literature cited," p. 631.

whether one or more species were involved; (2) to make cross inoculations with these cultures, to see whether or not there were any specificities which cause certain strains to infect definite hosts; (3) to make temperature studies of these fungi; (4) to determine the rôle of the microconidia in the life history of the genus Sclerotinia.

The material and cultures used in the following studies were all collected by the writer on the Chicago market, with the exception of a few strains, for which due credit is given in the footnotes. Material from practically every State has been collected and cultured. This has offered an admirable opportunity to compare strains of the genus Sclerotinia from all of the important truck-crop regions, and it is believed that the studies given here will be of special value in that such a wide geographical range and large variety of hosts were considered. The literature of the subject abounds with such excellent descriptions of the field diseases caused by Sclerotinia that it is felt hardly worth while to do more here than cite some of the more important articles. While the bibliography at the close of this article does not pretend to be complete, it is hoped it will furnish a working basis for any who may be interested in this subject.

ECONOMIC IMPORTANCE

Among the numerous fungi that are important in causing diseases in vegetables under transit, storage, and market conditions, members of the genus Sclerotinia rank well toward the head of the list. This fact has been especially noticeable since the establishment of the Food Products Inspection Service by the Bureau of Agricultural Economics of the United States Department of Agriculture. More than 30,000 inspections of perishable fruit and vegetable produce are now made at the receiving markets annually, and the inspectors' certificates on car-lot shipments covering a great number of vegetables show a surprisingly large loss due to decay caused by Sclerotinia species. In their studies of the market diseases of vegetables, Link and Gardner (*20*) adopted the term "watery soft rot" to describe the decay caused by *S. libertiana*, and said: "In the market it occurs on a wide variety of hosts. It is the prevalent rot at low temperatures, and is the most important storage rot of root crops." The type of decay produced varies somewhat with the different species and the different hosts, but there are some outstanding characteristics which are usually common to all. Chief among these is the soft, watery, odorless type of decay. If the host tissue is free from other contaminating organisms, it may become so completely penetrated by Sclerotinia mycelium that practically all of the water will be liberated without odor. In fact, the liberated juice of the host seems to increase the natural odor of the plant. A second characteristic is the abundant white cottony mycelium which is present on the surface of the host in a humid atmosphere, and the black sclerotia which are formed in the old mycelium in regions where the host tissues are partially consumed (pl. 1, B, C, H). The sclerotia are normally produced on the surface of the host, but in some instances under field conditions they are formed within the pith of stems, such as those of the potato, tomato, sunflower, and the seed stalks of lettuce (pl. 1, A). In such cases the sclerotia are often



Mycelial and sclerotial development of *Sclerotinia libertiana* and *S. minor* on various substrata

A.—Sclerotia of *S. libertiana* formed within the seed stalk of lettuce
B.—Luxuriant cottony growth of mycelium of *S. libertiana* on salsify root held in a moist chamber at room temperature
C.—Growth of *S. libertiana* on salsify under moderately dry conditions
D, E.—Mycelial and sclerotial development of *S. minor* on slices of parsnip and carrot, respectively
F, G.—Mycelial and sclerotial development of *S. libertiana* on slices of parsnip and carrot, respectively
H.—Sweet potato attacked by *S. libertiana*, showing development of large sclerotia

closely crowded together and take up most of the space in the hollow or semihollow regions.

In hampers of green beans and peas it is not uncommon to find the pods covered and joined together by the white, cottony mycelium, which grows luxuriantly in the humid atmosphere in the center of the container. This "nested" condition is common in wet seasons when field conditions are favorable for infection and development of the fungus. Hampers which contain only a few affected pods at time of shipment may show a relatively high percentage of infection and decay upon arrival at market. After selecting at random 42 cars of green beans, which on inspection at market showed Sclerotinia decay, it was found that the percentage of infection ranged from 5 per cent in some cars to as high as 75 per cent in others, the average for all the cars being 24 per cent. Random selection of 12 cars of green peas showed an average of 20 per cent infection and decay caused by Sclerotinia.

All of the common root crops, such as parsnip, salsify, turnip, rutabaga, and carrot are affected in about the same manner. The soft, watery, odorless decay is produced; and if the host tissue is white, as in parsnips, salsify, and turnips, a slightly pinkish color is often quite noticeable on the margin of the lesion, while the inner portion of the lesion is pale brown and water-soaked. The pink discoloration is also sometimes evident in celery, cabbage, and cauliflower.

In Table I are listed the most important truck crops that are subject to the attacks of Sclerotinia, and the States which ship the bulk of these products. This table shows that the fungus has a wide host range and that it is found wherever truck crops are grown. Several hosts of minor economic importance, such as cress, escarole, mangel, and others, are not included. The tomato fruit is also omitted because of the absence of an authentic record of the occurrence of Sclerotinia decay of tomatoes on the market, although the tomato fruit is very susceptible to decay when inoculated with all strains and species of Sclerotinia which the writer had under observation. In 1918, watery soft rot was reported in two cars of Florida tomatoes by the food products inspectors, but as it has never been found since then there may be some doubt as to the correctness of the diagnosis.

Tomatoes grown under glass are more liable to infection by Sclerotinia than those grown in the open field. McClintock (*21*) reported a fruit rot due to *Sclerotinia libertiana*, and Dickson (*11*) has reported a wilt of tomato plants in the greenhouse, on soil previously cropped to lettuce which had suffered an attack of "drop." A blossom blight of tomatoes in Albany County, N. Y., was also reported to the Plant Disease Survey in 1920 by H. W. Fitch. The causal organism in each of these cases was apparently *S. libertiana*. In April, 1923, W. A. Orton and A. C. Foster found tomato plants near Sanford, Fla., which were affected with a sclerotia-forming fungus, and material sent to the writer showed numerous small, irregular black sclerotia in the pith regions of the stems. Cultural and cross-inoculation work indicates that this organism is identical with *S. minor*, which is reported by Jagger (*18*) to affect lettuce in that locality.

TABLE I.—*Important crops affected with Sclerotinia decay, and the States which ship the greater amount of this produce*

	Alabama	California	Colorado	Delaware	Florida	Georgia	Illinois	Indiana	Kentucky	Louisiana	Maryland	Massachusetts	Michigan	Minnesota	Mississippi	New Jersey	New York	North Carolina	Ohio	Oregon	Pennsylvania	South Carolina	Texas	Virginia	Washington	Wisconsin
Asparagus		×																			×					
Bean	×	×			×		××			×	××		×		×	××	×	×		×		××	×	××	×	×
Broccoli	×	××	×		×		××	×	×	××	×	×	××××		××	××××	××××	×		×	×	×	×	××	××	
Cabbage	×	××			××××××		××	×			××		×××			×××	×××	×	×		×	×	×	××	××	×
Carrot			×	×			×	×			×		×			×	×	×	×			×				
Cauliflower							×			××			××	×		×	×	×	×			×				
Celery				×			×			×			×			×	×	×								
Chicory																										
Cucumber																										
Endive																										
Lettuce																										
Parsnip																										
Pea																										
Rutabaga																										
Salsify																										
Sweet potato																										
Turnip																										

Table II,[5] compiled from the reports of the food-products inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture, for 1920, presents in a concise form the damage sustained by that part of the carrot crop which was inspected in our principal markets. Practically half of the cars inspected showed more or less decay (watery soft rot) due to Sclerotinia. The range up to 100 per cent infection in advanced stages shows how serious the ravages of this fungus may be.

TABLE II.—*Prevalence of Sclerotinia watery soft rot of carrot as determined by food-products inspectors of the Bureau of Agricultural Economics, United States Department of Agriculture, 1920*

Origin of shipment	Number of cars inspected	Number of cars with decay	Average percentage of decay	Date of inspection	Market where inspected	Percentage of decay	Remarks
				1920			
California	7	3⅓	14	Mar. 31	St. Louis	12 to 15	
				June 30	Chicago	3 to 5	
				July 1	St. Paul	20 to 30	
				Aug. 9	New Orleans	10 to 15	One-third of car.
Illinois	4	4	52	Dec. 17	Boston	4	
				Dec. 20	Chicago	25 to 30	Large pits at stem.
				...do	...do	75 to 100	Advanced stage.
				...do	...do	90	Do.
Louisiana	2	2	55	Mar. 16	...do	80 to 90	
				June 17	Detroit	25	With Rhizopus rot, worst in upper tiers.
Mississippi	1	1	45	June 18	Minneapolis	40 to 50	Decay in tops.
New York	20	4	10	May 6	Washington	5	
				Nov. 1	New York	15 to 20	
				Nov. 9	...do	15	
				Nov. 13	Pittsburgh	3 to 5	
Ohio	2	1	22	Dec. 9	...do	20 to 25	
Oregon	2	2	20	Jan. 12	New Orleans	25 to 30	
				Feb. 9	Minneapolis	10 to 15	
Texas	3	2	20	May 4	St. Louis	2	
				May 28	Chicago	25 to 50	In tops.
Unknown	11	4	24	Feb. 16	New York	15	
				Mar. 17	Pittsburgh	15	
				Oct. 29	...do	15 to 20	With slimy soft rot.
				Nov. 30	Washington	35 to 60	With gray mold rot and Rhizopus rot.

Total number of cars inspected	52
Total number of cars with watery soft rot	23⅓

Similar figures for the lettuce crop of 1920 show that of 613 cars inspected, 108 had decay due to Sclerotinia. In 36 of these cars, between 75 and 100 per cent of the heads were infected. These heads are not usually a total loss, but any considerable amount of infection necessitates severe trimming before the product can be placed on the market. In the cars showing Sclerotinia decay that were shipped from the eight leading lettuce-producing States during 1920, an average of 54 per cent of the heads was affected.

Watery soft rot is also one of the most severe transit rots of celery. About half of the car-lot shipments which the Bureau of Agricultural Economics is called upon to inspect show more or less of this decay. In 1920 more than 365 cars were inspected, and of this number 207

[5] HASKELL, R. J., and WOOD, J. I. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1920. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Surv. Bul. Sup. 16, p. 271, table 100. 1921. (Mimeographed.)

were affected with Sclerotinia decay. The percentage of infected plants in these cars ranged from 1 per cent in some cars to 100 per cent in others, the average being approximately 50 per cent.

Sclerotinia libertiana occurs much less frequently on fruits than on vegetables. The writer has studied a strain from lemon,[6] which was known to cause "cottony rot," and also a culture from strawberry. Smith (*27*) concluded from his morphological and cross-inoculation work that the organism causing "cottony rot" of the fruits and attacking the twigs of oranges and lemons in the orchard is identical with *S. libertiana*. In the present work, the writer has been unable to find any differences between the strains just mentioned and many other strains obtained from vegetable crops. The sclerotia, apothecia, asci, and spore measurements are similar, and inoculation work has shown these strains to be pathogenic to all of the vegetables tried, except potato. It appears, therefore, that *S. libertiana* is not limited to the vegetable crops, but that the manner of growth of the host and the opportunities for infection are the important limiting factors.

A study of climatological, together with the inspectors' certificates issued by the United States Bureau of Agricultural Economics on car-lot shipments of perishable produce, show that a rainy season just before and during harvesting time is very favorable for Sclerotinia, and that crops are more likely to become infected under such conditions and decay during transit or storage. This is very well illustrated by a refrigerated car of Louisiana carrots inspected by the writer in Chicago. These carrots were wet and dirty and gave every evidence of having been dug in wet weather. They were inspected and accepted at loading point as a good product. The car was in transit only four days, and its refrigeration record (40° to 46° F.) was good throughout the trip, yet on arrival at Chicago careful inspection showed that more than 85 per cent of the carrots were affected with Sclerotinia decay. The tops were wet and badly decaying, and the roots showed watery lesions often covering over 25 per cent of their surface. This is also a very good illustration of the fact that products which appear healthy at loading time may be contaminated or may have incipient lesions which develop into serious decay in transit. There was a loss of approximately $900 on this car due to this decay. This is only one of the many instances that might be cited to show the swift destruction of vegetable produce caused by Sclerotinia under refrigeration and transit conditions that are ordinarily considered good.

MORPHOLOGY

A large number of cultures have been compared in an attempt to ascertain whether there were any morphological differences between the various strains of Sclerotinia collected, as shown by mycelium, sclerotia, apothecia, asci, spores, and microconidia.

In culturing fungi, it has sometimes been noticed that the type of inoculum used in making the transfer determines, to some extent at least, the type of growth and spore formation (*1*). In transferring from old cultures of Sclerotinia, the sclerotia are probably most often

[6] Original culture obtained from H. S. Fawcett, through courtesy of D. H. Rose.

used as inocula, while from young cultures the mycelium usually is transferred. The writer had never noticed any difference between cultures raised from sclerotia and those grown from mycelial transfers, but in order to get definite data on this point and to note in particular whether there was any tendency to change the manner or amount of microconidial production, or a tendency for other conidial forms to develop, the following experiment was conducted. Six cultures were selected, five of *S. libertiana* and one of *S. intermedia* (*24*). From these cultures, two sets of transfers were made. In the first set only mycelium and the associated microconidia were used as inocula, while in the second set care was taken to transfer only sclerotia. These two sets of cultures were grown at room temperature in diffused light, and were transferred, in the manner just described, every month for nine successive months. Notes were taken on each successive generation at the end of one month's growth, in an effort to discover any differences in growth and development between the cultures from the sclerotial and those from the mycelial transfers. At the close of the period there was nothing to indicate that it would be worth while to continue the study. The results of this experiment can be summarized by saying that the chief difference noted was in the rate of growth. The mycelial transfers grew much faster immediately on being placed on new agar, consequently producing more vegetative growth and forming sclerotia sooner than the cultures arising from the sclerotial transfers. The slower-growing sclerotial plantings, however, were comparable to the other transfers by the end of the month. There was no tendency to change from the normal way of growing in either series, and no more sclerotia or microconidia were produced than usual.

EFFECT OF TEMPERATURE AND CULTURE MEDIA ON SCLEROTIA

Cultures of Sclerotinia grown at room temperature maintain approximately the same relative size of sclerotia for a given species, provided a suitable medium is used. In cultural studies, however, the writer has often noted that the relative size of the sclerotia of different species does not remain the same when extremes of temperature are involved. In general, when the temperature is near the minimum growing point for the fungus, the sclerotia have a tendency to be larger than the normal for room temperature. This is particularly so in the small sclerotial forms. *S. intermedia* on potato dextrose agar at 5 to 7° C. has been observed to form sclerotia 2 to 4 mm. in diameter, while the normal for this species is 2 mm. at 20°. On the other hand, sclerotia grown near the maximum for the fungus have a tendency to be smaller than normal for room temperature. Sclerotia of *S. libertiana*, which usually average about 4 mm. in diameter at 20°, have ranged from 1.5 to 4 mm., with an average diameter of 2.5 mm. when grown on the same medium at 30°. *S. intermedia* grows very poorly at this temperature and the sclerotia formed vary from 1 to 2 mm. in diameter.

Changes in the size of the sclerotia have also been noted in cultural studies when apparently other factors than temperature were involved. A Sclerotinia was isolated from parsnip which produced a scant mycelium with numerous very small sclerotia. The first isolation plate and the first transfer to tubes of potato-dextrose agar

gave sclerotia which would average less than 1 mm. in diameter. After several transfers on potato-dextrose agar, the sclerotia were 2 to 5 mm. in diameter, with an average diameter of about 3 mm. This leads one to suspect that the fungus had become better adapted to the artificial medium. Gilbert and Bennett (*14*) found in their study of *Sclerotinia trifoliorum* Erik. that large sclerotia were produced on favorable media such as potato plugs, while on unfavorable media the sclerotia were small. This undoubtedly explains why the sclerotia on the host plant are often quite different from those of the same fungus when grown on artificial media. From these observations it is quite evident that while the size of the sclerotia may be useful in separating species, this character can not be depended upon unless the influence of temperature and culture media are taken into consideration.

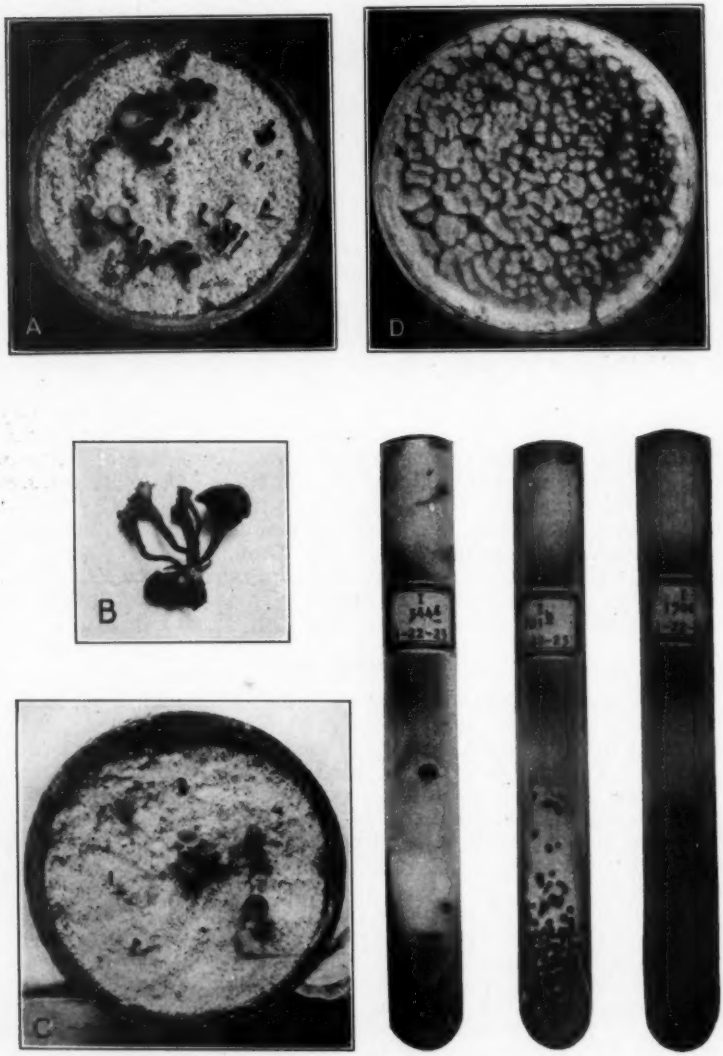
The sclerotia of *S. minor* and *S. intermedia* have showed constant differences from those of *S. libertiana* throughout all experiments. In all cultures of the large sclerotial type there were no appreciable differences in the sclerotia of the strains studied that could not be attributed to growing conditions. As it is fair to compare only sclerotia that have been grown under exactly parallel conditions, the following data will suffice to show the relative size of sclerotia of the strains of Sclerotinia under consideration. On plate cultures of oat-meal agar, the large sclerotial strains produced sclerotia ranging from 2.5 to 6 mm. in diameter, averaging 3.5 mm. In similar cultures of *S. minor* the range was 0.5 to 1 mm., average 0.78 mm. in diameter, while the sclerotia of *S. intermedia* ranged from 1 to 3 mm. in diameter, with an average of 2 mm. These species cultured on tubes of potato agar are shown in Plate 2, E, F, and G.

APOTHECIAL PRODUCTION

Laboratory experiments show that no definite period of dormancy is necessary to prepare sclerotia for production of apothecia. Sclerotia held in a dry state for several months do not germinate any more readily than those of the same kind which are one or two months old. No advantages have ever been observed from chilling or alternating the temperatures at which the sclerotia were held. There are, however, differences which are traceable to seasonal or ecological conditions, such as the influence of light, temperature, and moisture.

The development of apothecia may be divided into two phases. The first embraces the germination of the sclerotium and sending forth of the hornlike stalks; the second involves the development of the funnel-shaped top and its ultimate expansion into the disklike cap. Experiments show that germination of the sclerotia is dependent upon proper moisture and temperature conditions, while the main factor for the development and full expansion of the apothecium is light.

Best results in the laboratory have been obtained by planting mature sclerotia 5 to 10 mm. deep in sterile sand in an ordinary porous flower pot (pl. 2, A and C). Four-inch pots placed in saucers or halves of Petri dishes filled with sterile water have been found satisfactory if they are placed under bell jars. The use of sterile pots, sand, and water has been necessary in most instances in order to



Some developmental stages in the life history of Sclerotinia

A.—Apothecia of *S. libertiana* grown in flower pots of sterile sand
B.—Malformed apothecia of *S. libertiana*, produced under unfavorable growing conditions
C.—Apothecia of another strain of *S. libertiana*. Compare with A
D.—Plate of potato-dextrose agar showing numerous colonies of growth from germinating ascospores. Plate inoculated by being held over a "shooting" apothecium
E, F, G.—Parallel cultures of *S. libertiana*, *S. intermedia*, and *S. minor*, respectively, grown on potato agar

avoid the interference of algal growth on the surface of the sand and on the edges of the pots. These pots should be placed in rather strong diffused light. Those placed in the north and west windows of the laboratory have produced apothecia abundantly during the late winter and spring months.

Although sclerotia have been planted in sand during practically every month of the year, by far the greatest number have germinated and produced normal apothecia during the months from January to April inclusive (Table III). Since the water was administered artificially in all instances, this factor may be considered constant, thus leaving the variable factors of light and temperature to be determined. No way of sharply separating the influences of light and temperature has been found. Nevertheless, since sclerotia have been germinated in the dark when they were held at 18° to 22° C. during the unfavorable summer months and whereas they did not germinate in the dark at laboratory temperatures (22° to 30°) during this time, it seems safe to assume that temperature was the more important limiting factor.

TABLE III.—*Germination of sclerotia of Sclerotinia species in sand at laboratory temperature*

SCLEROTINIA LIBERTIANA

Source of culture	Date of culture	Date of planting sclerotia	Date of germination	Apothecia			
				Number started	Number matured	Diameter	Height above sand
						Mm.	*Mm.*
Bean	Jan. 4, 1922	Oct. 5, 1922	Feb. 2, 1923	6	6	3	3 to 6
Carrot	Aug. 9, 1921	Mar. 9, 1922	Apr. 4, 1922	3	3	1 to 3	4 to 8
Do	July 14, 1921	do	Apr. 10, 1922	3	3	3 to 5	4 to 6
Cauliflower	July 1, 1922	Oct. 6, 1922	Feb. 10, 1923	1	1	7	10
Celery	Aug. 1, 1921	Jan. 30, 1922	Feb. 20, 1922	15	10	2 to 6	5 to 12
Chicory	Jan. 25, 1922	Oct. 6, 1922	Mar. 15, 1923	4	1	2	4
Endive	July 28, 1921	Oct. 7, 1922	Nov. 14, 1922	9	2	4	5
Lemon	Oct. 10, 1922	Nov. 14, 1922	Jan. 30, 1923	15	5	4 to 6	4 to 8
Lettuce	June 15, 1921	Oct. 5, 1922	Feb. 21, 1923	8	7	3 to 6	5
Do	Direct from host.	Feb. 2, 1923	Mar. 5, 1923	69	8	5 to 13	4 to 10
Do	June 2, 1921	Jan. 30, 1922	Feb. 17, 1922	6	3	4 to 5	4 to 6
Parsnip	July 28, 1921	Mar. 9, 1922	Apr. 15, 1922	14	12	4 to 7	6 to 8
Salsify	do	do	Apr. 22, 1922	6	6	3 to 6	5 to 8
Strawberry	Oct. 8, 1920	Feb. 24, 1921	May 12, 1921	3	1	4	5
Sweet potato	Apr. 1, 1922	Nov. 8, 1922	Jan. 9, 1923	16	3	3 to 4	5 to 7
Do	Direct from host.	Dec. 28, 1922	Feb. 7, 1923	88	14	3 to 5	3 to 6
Turnip	June 12, 1921	Jan. 30, 1922	Feb. 20, 1922	3	3	5	5 to 8

SCLEROTINIA INTERMEDIA

Salsify	Sept. 14, 1920	Apr. 10, 1921	Apr. 28, 1921	18	10	2 to 4	5 to 8

Continued warm weather seems detrimental to apothecial production, but apothecia will form when the temperatures alternate between night and day, providing the extremes are not too great. This is shown by the development of normal apothecia in the winter months of December, January, and February, when the day temperature ranged between 20° and 22° C. and the night temperature between 13° and 18°.

Experiments do not show that any definite time is necessary between the planting of sclerotia and their germination. Sclerotia planted in

August have germinated in January, and sclerotia planted in April have germinated and produced apothecia in April. The shortest time between planting and germination was 18 days, while the longest was 141 days. From this it seems safe to assume that the time of apothecial production depends entirely upon ecological conditions such as moisture, temperature, and light. Variations in the length of time between date of planting and date of germination, as shown in Table III, must be attributed to these ecological factors rather than to differences between strains of *S. libertiana*.

Small sclerotia are especially susceptible to decay when the pots are kept too moist, and for this reason it is usually much more difficult to obtain apothecia of the small sclerotial forms than from those of the *S. libertiana* type. Various investigators have found that the sclerotia of *S. libertiana* will remain alive in dry state for several years, but it is usually agreed that in nature most of them decay during the first year. In this connection it is interesting to note, however, that Pollock (*23*) has given evidence to show that the sclerotia of *Sclerotinia cinerea* (Bon.) Schrot. may remain alive for 10 years in fallen mummified fruits.

As noted by Stevens and Hall (*29*), the light factor has been found to be especially prominent in stimulating the full development and expansion of the disk-shaped apothecium. Small, hornlike outgrowths are often found growing from sclerotia in old test-tube cultures which have been stored in the dark, but the writer has never known of one to expand into a normal apothecium. These aborted apothecia are quite common in sand-pot cultures that have been held in weak light. In some experimental pots used by the writer, more than 100 apothecial stalks have started out normally and then failed to expand presumably because of unfavorable light conditions. When these pots were placed in good light, the apothecia which started out subsequently developed in a normal manner. Abnormal and freakish apothecia are often formed when unfavorable growing conditions alternate with conditions favorable to growth. This stopping and resuming of development often leads to such freaks of apothecia as shown in Plate 2, B.

SINGLE ASCOSPORE ISOLATIONS

When apothecia are allowed to mature and fully expand under a bell jar, it is comparatively easy to obtain single spore cultures. A smokelike cloud of ascospores is discharged almost immediately upon a change of humidity brought about by lifting the bell jar. This cloud of ascospores is visible even when only one apothecium discharges, owing to the fact that even a moderate-sized apothecial cup may contain an enormous number of spores. Stevens and Hall (*29*) estimated that one cup may produce 31,000,000 spores. If a large number of apothecia are ripened at the same time and held under cover so that their discharge can be controlled, it has been found possible to photograph this cloud of spores as it rises into the air.

Dickson and Fisher (*12*) devised a method by which an excellent photograph of the spore-cloud emanating from apothecia of *S. libertiana* was obtained. By holding a Petri dish containing a thin layer of nutrient agar inverted over a discharging apothecium, a large number of the spores are shot against agar surface. The

spores thus caught on the agar film can be seen with the low power of the microscope and marked by a dot of ink on the bottom of the Petri dish. By choosing a thinly seeded portion of the plate for marking the spores, and by using reasonable care, a small portion of agar above the marked location of the spore can be lifted out without fear of getting more than one spore. If, however, the agar is cloudy and there is doubt as to the location of the spores, it has been found practical to hold the Petri dish culture for 12 hours, or until short germ tubes have started. When observations are made early it is not difficult to locate single germinating ascospores.

ASCI AND SPORES

The spore and ascus measurements are very important in distinguishing between species of the genus Sclerotinia, and for that reason a large number of measurements from numerous cultures have been made. The average measurements of typical large sclerotial strains of Sclerotinia from important host plants are listed in Table IV. For the sake of comparison the corresponding measurements of *S. minor* (*18*), *S. ricini* (*15* and *16*), and *S. intermedia* (*24*) are also given. This table shows a striking similarity in spores and asci of the 28 strains belonging to the large sclerotial type herein listed. While there is a slight variation between strains from different hosts, this difference is no greater than that within strains from the same host or the variations even within a given strain. The average measurements of asci ranged from 8.2 to 10.2 microns in width and from 125.4 to 160.4 microns in length, the mean of the averages of all large sclerotial strains being 9.2 by 144.3 microns. The spore averages ranged from 5.9 to 7.3 microns in width, and from 11.7 to 15.1 microns in length, their mean being 6.6 by 13.3 microns.

TABLE IV.—*Average measurements in microns of spores and asci of several strains of Sclerotinia libertiana as compared with each other and with S. intermedia, S. minor, and S. ricini*

S. LIBERTIANA

Culture No.	Host	Asci	Spores
822	Bean	9.5×156.4	6.7×12.7
283	Carrot	8.2×144.7	7.0×14.1
763a	do	9.2×144.4	6.7×13.8
760	Cauliflower	8.6×142.8	6.6×13.2
215a	do	9.9×155.0	7.0×14.0
187b	Celery	9.4×146.3	6.8×13.4
695	do	9.3×143.6	7.3×12.4
8/24	do	9.5×144.1	6.8×13.3
738	do	9.5×151.3	6.9×13.3
345	Chicory	9.0×133.0	6.0×11.9
749	Endive	9.8×145.9	6.9×13.3
612	Lemon	9.4×144.6	6.6×13.1
344	Lettuce	8.8×130.4	6.6×13.2
693	do	8.2×138.1	5.9×12.6
705	do	8.6×138.3	6.2×12.8
415	do	9.1×143.2	6.4×13.0
719	do	9.2×155.7	6.8×13.5
1466	do	10.2×158.5	7.0×14.0
349	do	9.9×150.8	7.1×13.5
261	Parsnip	8.7×138.3	6.8×12.9
744	do	9.0×136.9	6.5×13.9
732	Salsify	9.4×160.4	7.0×14.4
819	do	8.6×141.8	6.7×14.0
820	do	8.6×125.4	6.2×13.2
833	do	9.6×139.8	6.9×11.7
834	do	9.3×145.1	6.2×15.1
177	Sweet potato	9.0×142.8	6.4×13.0
1567	do	9.8×152.7	6.4×12.6

TABLE IV.—*Average measurements in microns of spores and asci of several strains of Sclerotinia libertiana as compared with each other and with S. intermedia, S. minor, and S. ricini*—Continued

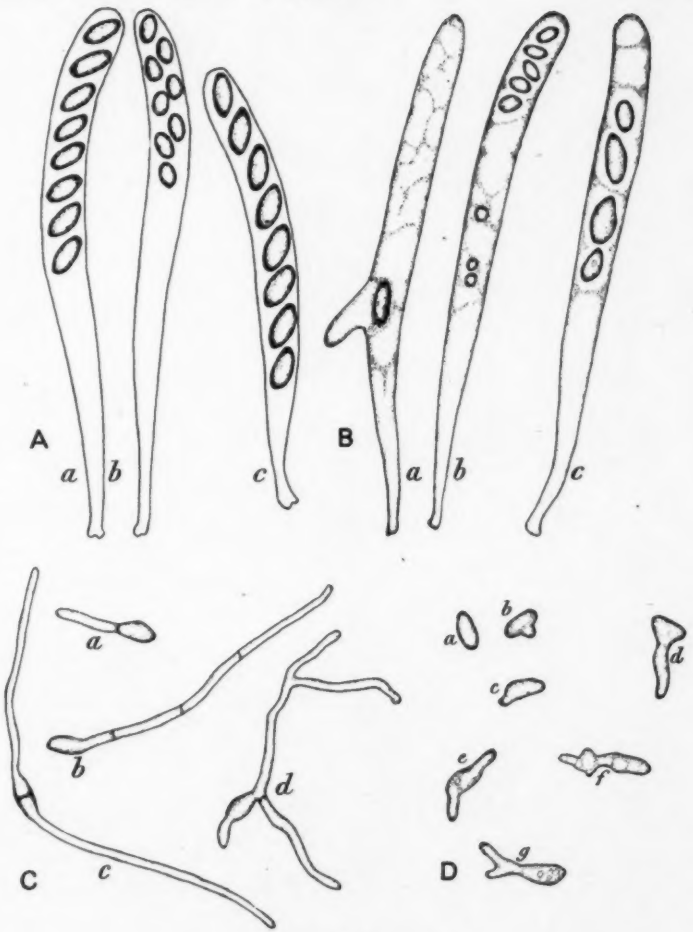
Culture No.	Host	Asci	Spores
	S. INTERMEDIA		
303	Salsify	7.5×127.0	4.9×12.7
	S. RICINI		
697	Ricinus	8.0×80–100	4.5×9–12
	S. MINOR		
1500	Lettuce	8.9×141.0	7.0×14.1

Certain minor morphological variations of asci and spores are shown in Plate 3, A, *a*, *b*, *c*. These asci are each from a different strain of *S. libertiana* and they represent rather wide variations in size as well as in shape; but as more spores and asci were measured and studied the writer became more and more skeptical as to the importance of these variations. The stage of maturity of the apothecia and their variations in development due to the reactions to moisture, temperature, and light conditions are responsible for many changes in form and size of normal spores and asci, even of the same strain, as well as such unusual forms as shown in Plate 3, B, *a*, *b*, *c*, which came from the malformed apothecium shown in Plate 2, B.

The apothecial measurements of several large sclerotial strains of Sclerotinia and of one strain of *S. intermedia* are included in Table III. The mature apothecia that were developed in good light usually attained a diameter of about 5 mm., but in exceptional cases some grew 10 to 13 mm. in diameter. The apothecia of *S. intermedia*, for the most part, range from 2 to 4 mm. in diameter. The average height of the apothecia above the surface of the sand in pot cultures was between 6 and 7 mm. Differences in depth at which the sclerotia were planted as well as fluctuations in light, are responsible for so much variation in these dimensions that they can be considered only as indicative of the measurements that may ordinarily be expected.

MICROCONIDIA

Several groups of fungi, during some phase of their life history, develop sporelike bodies which on a morphological basis, might be called spores (pl. 4, H). Lack of definite knowledge concerning their functions, however, has caused considerable uncertainty as to the terminology to be used in speaking of these morphological units. In many ascomycetes these bodies are formed copiously by either intercalary or acrogenous abjunction and are called gonidia. They are often associated with ascocarps or other fruiting bodies and have been referred to as rudimentary spores. The spermatia of other fungi also seem to differ little if any from many acrogenously formed



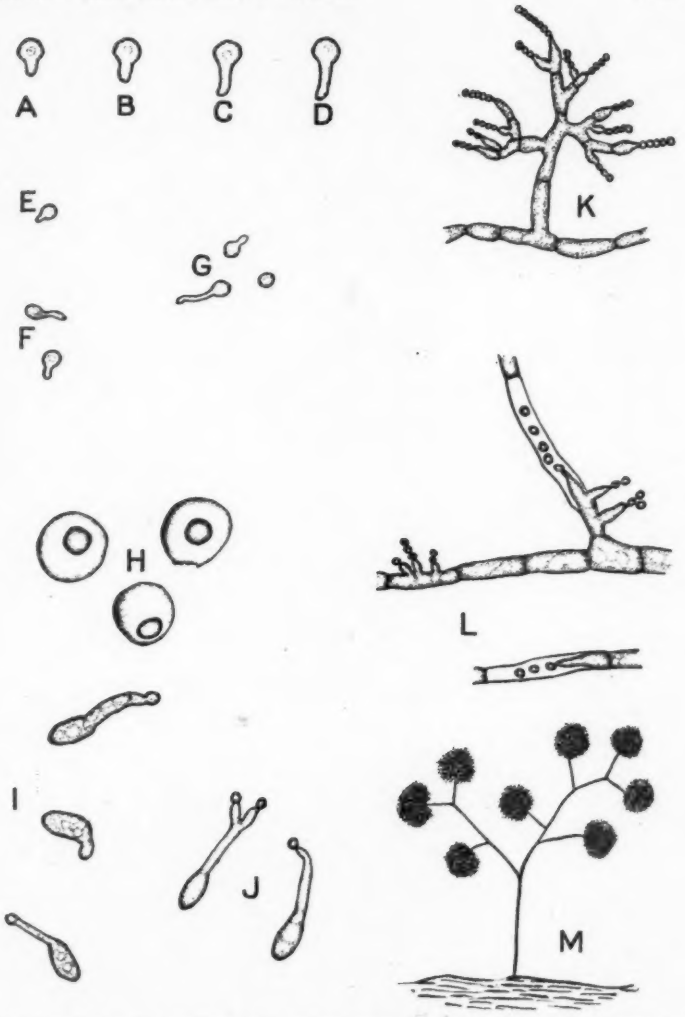
Asci and spores of *S. libertiana*

A, *a*, *b*, and *c*.—Showing variations in form and size of the asci and spores in different strains of *S libertiana*

B, *a*, *b*, and *c*.—Malformed asci and spores from deformed apothecia similar to those figured in Plate 2, B

C, *a*, *b*, *c*, *d*.—Ascospores of *S. libertiana* germinated in distilled water at 20° C.

D, *a* to *g*.—Ascospores of *S. libertiana* germinated in distilled water at 3 to 4° C.



Stages in the formation and germination of microconidia. All drawings were made with the aid of a camera lucida

A, B, C, D.—Stages in the germination of a single microconidium of *S. libertiana* as observed under the microscope, in a hanging drop of sterile distilled water, at room temperature (20° to 24° C.). Drawings made at 3, 4, and 7 p. m. (8/10/20) and at 8 a. m. (8/11/20), respectively. No further development was made after this date

E, F.—Germinating microconidia of two other strains of *S. libertiana*, in hanging drops of water distilled in glass

G.—Germinating microconidia of *S. intermedia* in a 5 per cent sugar solution at 20° C.

H.—Microconidia of *S. intermedia* highly magnified under oil

I.—Ascospores within an overripe apothecium germinating and forming microconidia on the tips of the term tubes

J.—Microconidia of *S. libertiana* formed on the tips of the germ tubes of germinating ascospores in distilled water

K.—Chainlike formation of microconidia upon the lateral branches of vegetative hyphae

L.—Endogenous formation of microconidia in turnip-broth cultures of *S. libertiana*

M.—Diagrammatic sketch of the botrytislike formation of microconidia of *S. trifoliorum*, as seen growing on agar plate, under the binocular

spores. In fact, the only difference seems to be in the germinating power. The bodies which germinate are called spores and those that do not germinate, or have never been observed to germinate, are called spermatia, or gonidia.

In 1918 Brierley (*8*) was successful in germinating the small gonidia or microconidia of *Botrytis cinerea*. He found that these microconidia germinated in form 24 to 48 hours at 17° to 23° C. when placed in water or a nutrient medium. In water the growth soon stopped, but in nutrient media it continued until a normal Botrytis growth was obtained. It was concluded, therefore, that the microconidia were a normal developmental stage in the life history of *Botrytis cinerea*. After studying the conditions governing microconidial formation, Brierley considered the age of the fungus to be the chief determining factor. With few exceptions, the microconidia were not found in cultures less than 30 days old. The amount of available food seemed to be next in importance, since it influenced the quantity but not the kind of spore production. Light, moisture, and temperature apparently had no particular influence upon this type of spore formation.

The microconidia of the genus Sclerotinia are produced in various ways. Short, lateral branches arising from the vegetative mycelium or tips of the normal hyphae may produce these sporelike bodies in great abundance (pl. 4, K, L, and M). Apparently Sclerotinia can produce microconidia at all stages in its life history when proper conditions are furnished. They have been found on the surface of sclerotia, on the disks of overmature apothecia which were held in a humid chamber, and on the tips of germ tubes where ascospores have germinated within the apothecium and in distilled water cultures (pl. 4, I and J).

Microconidia which are of endogenous formation have also been noted in cultures grown in rutabaga broth (pl. 4, L). Sporogenous hyphae form within some of the older vegetative cells and microconidia are cut off from the tip of the flask-shaped sterigmata which terminate these young hyphae. Vegetative cells have been found which contained large numbers of these small endoconidia. The manner of spore formation just described is practically identical with that figured and described by Beauverie and Guilliermond (*4, pp. 281, 315*) for *Botrytis cinerea* when grown in sterile distilled water.

The microconidia of the genus Sclerotinia are very similar to those of Botrytis, and are produced in extremely large numbers on media which are unsuitable for the vegetative growth of the fungus. Under such conditions, very scant mycelium is produced, and these small sporelike bodies are formed within a few days. On cultures of standard nutrient media, such as potato, bean, or oatmeal agar, most strains of Sclerotinia do not begin microconidial production until they are 20 to 30 days old.

So far as observed, the amount of available food seems to be the chief factor in determining the time of microconidial production. Abundant food of a suitable kind stimulates vegetative growth greatly, and the microconidia are not produced until the fungus has apparently used up all or most of the available surface food. As noted above, this stage is reached in most test-tube cultures about the twentieth day. Microconidia are seldom found on the natural

host plants. This is chiefly due to the fact that they are not formed until the host tissue is almost totally consumed, and when plants reach this condition they are usually thrown away without examination.

More than 200 cultures have been under observation for 3 years, and all have at some time produced microconidia in greater or less abundance. Some strains normally produced more than others, but this occurred on the general stock media, potato-dextrose agar, which was manifestly less suitable for vegetative growth of some cultures than others.

The method and time of production of these small microconidia leads one to suspect that they either function now as true spores in the life history of the fungus or that they have done so in the past. Working on this latter theory, the writer studied a large number of cultures from a wide geographical range and a large number of hosts, in the hope that some cultures would be in such a stage of adaptation or evolution that their microconidia would still be functioning. In all cultures collected the size, shape, and color of the microconidia, and the time and manner of sporulation, were essentially the same. No peculiarities or distinctions of any kind could be noted that would indicate that one culture was more likely to be viable than another.

While potato agar plus 2 per cent dextrose is a good culture medium for most Sclerotinia species, the addition of the dextrose seems to have a decidedly inhibiting influence on microconidial production. Few if any microconidia are formed in potato-dextrose agar cultures 20 to 30 days old, whereas on potato agar they are formed in great abundance at this age. On 20 per cent dextrose agar, a moderate growth of mycelium and a few sclerotia are produced, but no microconidia are found even in cultures 30 days old. In contrast with these results, cultures on plain potato agar and on Pfeffer's agar, held at room temperature, have formed some microconidia within 10 days, and plates of pure gelatin inoculated with *S. libertiana* have developed in 10 days a very scant, loosely appressed mycelium which bore prodigious numbers of microconidia on the surface as well as on the submerged mycelium.

GERMINATION STUDIES

While the presence of microconidia in Sclerotinia cultures has been noted by many writers, no one, as far as the writer is aware, has ever seen them germinate. The fact that they are borne late in culture, and when found look like a contamination, may account for lack of work on this particular phase of the life history. During the past three years the writer has devoted a great deal of time and study to the attempt to determine the rôle of the microconidia in the life history of the genus Sclerotinia.

The apparent de novo origin and rapid spread of watery soft rot in transit in such crops as lettuce, celery, cabbage, carrots, etc., has often suggested that some spore form was responsible for the primary field infection and for spreading the fungus from one plant to another during the transit period. A large part of this apparent spreading is undoubtedly due to ascospore infection from the field, but since even a small lesion in advanced stages of decay may bear innumerable

microconidia on the vegetative mycelium, it seemed of first importance to determine whether these microconidia were capable of germination. In this study the methods employed were, in general, very similar to those used by Duggar (*13*) in his studies on the germination of certain fungous spores. The extremely small size of the microconidia and their lack of color make working with them very difficult. In order to use the high power of the microscope, it was necessary to make practically all study with hanging-drop cultures. While miscellaneous experiments were conducted with numerous cultures from time to time in attempts to get the microconidia to germinate, the greater part of the work was centered about 50 strains of Sclerotinia, including *S. libertiana*, *S. intermedia*, *S. ricini*, and *S. trifoliorum*. These cultures represented a host range of 30 plants which are commonly affected by Sclerotinia and which were obtained from widely separated parts of the United States.

The more important nutrient solutions and materials used in the germination studies, testing out the ability of the microconidia of Sclerotinia species to germinate, were:

Distilled water, water distilled in glass, tap water, sterile tap water, fresh rain water, sterile rain water, liquid exudate from newly forming sclerotia, freshly cut slices of carrot roots, leaves of head lettuce, sterile distilled water with bits of fresh carrot tissue, celery tissue, and lettuce tissue added separately, freshly expressed juice of salsify roots, carrot roots, beet roots, turnip roots, rutabaga roots, lettuce leaves, celery stalks, ripe tomato fruit, lemon fruit, alfalfa stems, clover stems, decoctions of each of all these plants, soil decoction, ant decoction, beef bouillon, prune juice, sugar solutions, standard nutrient-salt solution, Pfeffer's nutrient solution, liquid gelatin, hard gelatin, potato agar, potato-dextrose agar, prune agar, and oat-meal agar.

In practically all experiments, hanging drops of the nutrient solution containing microconidia were made in Van Tieghem cells, and held at room temperature in diffused light. In most instances duplicates were placed in an incubator at a temperature of approximately 8° C. In many cases microconidia were separated from a suspension of mycelium and spores by filtering through filter paper. The filtrate containing the microconidia was then used to flood over agar and gelatin plates and to spray on host plants and on parts of their organs. As a rule, cultures were held for one week and examined daily for germination.

In the first hanging-drop cultures, tap water, distilled water, and rain water were used as a medium in which to test out the germination of microconidia. But such poor results were obtained (pl. 4, A, B, C, and D) that in order to find out whether substances present in the tap water or traces of copper in the distilled water were acting as toxic agents these were distilled in glass and used as media in further studies. These results, however, were no more favorable than the others. Short germ tubes started in several cultures within 12 to 24 hours, but the growth then stopped and no changes in temperature or light conditions were found that would induce further development (pl. 4, E and F).

From the results obtained with the water-culture experiments it appeared that microconidia belong to that class of spores which require a nutrient or some stimulating agent in order to germinate well. In view of the fact that Brown (*10*) found a decided stimulating effect produced by the volatile substances arising from bruised host

tissues in spore-germination studies of *Botrytis cinerea*, several experiments were devised in order to test similar effects on microconidia. To be sure that there were no small bits of mycelium in the spore suspension, the microconidia were filtered through filter paper. The filtrate, free from mycelium, was then used to inoculate freshly cut slices of carrot root and to be sprayed on leaves of head lettuce. The inoculated plants were held in moist chambers, at room temperature, in diffused light, and examined daily for symptoms of infection. Parallel with these inoculation experiments, several hanging-drop cultures were made in van Tieghem cells by adding bits of carrot-root tissue to the drops so that growth might be observed with the microscope.

The results of these experiments and others of similar nature were disappointing. No infection of the host occurred, and only an occasional short germ tube could be found in the hanging-drop cultures. Many hanging-drop cultures of microconidia in various dilutions of the freshly expressed juices of the host plants listed in a previous paragraph were made from time to time. In most of these cultures germination either was very feeble or there was none whatever. When germination took place, the germ tubes developed 2 or 3 spore diameters (7 to 12 microns) in length within 48 hours, and then stopped growth. Cooked broth or decoctions in general gave a higher percentage of germination than fresh juices of the same plants, but in none could development be induced when single spore cultures were attempted. Consequently, no culture has ever been obtained by the writer which can definitely be traced to a single germinating microconidium.

In addition, sugar solutions of various strengths, as well as the standard nutrient salt solution as used by Duggar (*13*), and Pfeffer's nutrient solution, were used as media. In these, as in previous experiments, only a small percentage of the spores produced germ-tubes (pl. 4, G). These ceased growing after 48 hours, and no changes in light or temperature conditions were found that would induce them to further activity.

The only cultures obtained from microconidia during these studies were from plantings made on potato and prune agar plates. Microconidia suspended in sterile distilled water were filtered through filter paper, which was found to remove particles of mycelium, and drop plantings of the filtrate were then made on hard agar in Petri dishes. No growth was visible to the unaided eye for two days, but on the third day mycelial development was observed in all plantings. Within one week these cultures made a normal growth of mycelium and sclerotia which appeared similar in every way to the usual cultures of *S. libertiana*.

It appears, therefore, that conditions must have been favorable in these experiments for the full development of the microconidia, although, as far as the writer is aware, these conditions were no different than those under which many other unsuccessful germination tests were made.

In order to test the microconidia for a reaction to oxygen, a series of hanging-drop cultures was made in the usual way. A drop of distilled water with dioxygen added as a source of oxygen was used as a medium. Cultures were made using the following percentages

of normal dioxygen (3.75) : 0.5, 1, 2, and 5 per cent. The microconidia were taken from a culture 19 days old. Control hanging drops of microconidia in sterile distilled water were run parallel with the above experiment. Daily microscopic examination was made of all cultures for two weeks, but no germination was obtained. The controls as well as all other cultures remained inactive. It appears, therefore, that lack of oxygen probably is not the cause of poor germination.

So far as the writer has been able to ascertain, temperature and light conditions are not the limiting factors, either in the formation or germination of microconidia. To test out the age factor, germination studies have been carried on from cultures which varied in age from 4 days up to 157 days. The microconidia which gave most favorable germination were usually from cultures 20 to 30 days old, although apparent success was obtained from cultures as young as 7 and as old as 132 days. Because of the difficulty of definitely determining the time when the microconidia are formed, it is impossible to determine at just what age they are most likely to germinate. All observations along this line indicate that the microconidia are mature as soon as they are formed. No change in color, size, or shape is noticeable as the cultures age.

In all the experiments devised to test the germination of microconidia, approximately 10 per cent were successful. From these germinated microconidia, only two cultures ever developed a vigorous vegetative growth that could be recovered. These cultures were obtained from agar plates, and the greater percentage of other successful studies were obtained from artificial or synthetic media. In view of this and the fact that no infection of host plants or their fresh tissues was obtained, it seems highly probable that the microconidia of the genus Sclerotinia do not play an important rôle in the life history of this group of fungi and do not cause infection in the field, transit, or storage.

INFECTION STUDIES

Artificial infection of a suitable host is readily obtained, under favorable conditions, by inoculation with sclerotia, or vigorously growing mycelium (pl. 1, D, E, F, and G). The writer's experiments clearly indicate that the limiting factors in obtaining infection are moisture and temperature. More inoculation experiments have failed because of lack of water during the first penetration of the host tissues than from all other causes.

Wounds are not necessary for infection, but undoubtedly wounded surfaces with their exuding cell contents offering readily available food greatly facilitates the progress of the fungus. The succulent plants and plant parts are penetrated most rapidly, while the woody tissues are much more resistant. This indicates that the mechanical make-up of the plant tissues plays a part in resistance to Sclerotinia infection. Boyle (7) came to the conclusion that *S. libertiana* effected rupture of the host tissues solely by mechanical pressure. He found that the mycelium and appressoria of *S. libertiana* growing in turnip juice were surrounded by a mucilaginous sheath which fixed and held the "infection hyphae" to the cuticle of the host. He also noticed that the hyphal tips in contact with resistant material showed different staining from normal and that the infection hyphae

arose from these tips. Similar observations have been made on other fungi. Brown (*9*) found that *Botrytis cinerea* did not produce a substance in the germ tube which was capable of dissolving cuticle. Blackman and Welsford (*6*) found later that in the early stages of infection, *B. cinerea* effected its entrance into bean leaves solely by mechanical pressure exerted by the germ tube. It should be noted in this connection, however, that although first penetration of the cuticle is made by mechanical pressure, Boyle (*7*) found that as soon as the infection hypha gets within the cellular tissues, a chemical action takes place and cells die some distance beyond the invading hyphae, thus suggesting the secretion of an enzyme.

INFECTION BY MEANS OF ASCOSPORES

It has heretofore been the general belief that it was necessary for ascospores of the genus Sclerotinia to fall upon some dead organic matter and first establish themselves saprophytically in order to gain enough vigor to attack living plants. The work of Stevens and Hall (*29*) on *S. libertiana* and that of Beach (*3*) on *S. minor* indicated that direct infection by ascospores seldom, if ever, occurred. Pethybridge, in his investigations of the stalk or sclerotium rot of potatoes produced by *S. sclerotiorum*, writes (*22*):

> Although these spores or, perhaps, more correctly speaking, their germ tubes, are incapable of entering normally vigorous green potato foliage and stalks, they are evidently capable of causing infection of the foliage when it is yellowing and ripening off, and probably are also capable of infecting the stalks at the scars remaining when the older leaves have fallen. During the past season at Clifden circumstances were very favourable for observations on the mode of attack of this fungus, and in very numerous instances it was abundantly clear that infection occurred first on one of the older yellowing leaves from which the fungus passed directly to the stalk which bore it.

The writer has been able to show in laboratory experiments that freshly cut slices of turnip, carrot, and lettuce leaves held over "shooting" apothecia will become so severely infected that the whole surface of the host will be covered with white, cottony mycelium, and the tissues reduced to a watery mass within three to five days. The rate of growth is comparable with that produced by a direct transfer of a vigorous mycelium from plate cultures. Successful inoculation has also been obtained by spraying an ascospore suspension in distilled water upon individual leaves of head lettuce. In this experiment, however, the growth of the mycelium was so general over the surface of the leaves that it was impossible to locate the original points of infection. These experiments show that ascospores can at least infect freshly-wounded tissues if not living cells.

CROSS INOCULATIONS

As just pointed out, a study of the cultural characteristics and morphology of the various Sclerotinia cultures obtained from market produce indicated that the same strain of Sclerotinia attacks a wide range of hosts. Cross-inoculation tests were made to determine whether there were any physiological races or strains. In general, the experiments were made at room temperature, in diffused light. Fresh host or host tissues were obtained from the market and sterilized in mercuric chloride (1 to 1,000) for a few minutes, and then rinsed in sterile water. Inoculations were made in wounds and on unwounded

surfaces, and the specimens were held in large sterile moist chambers. Vigorously growing mycelium a week old on a small piece of potato-dextrose agar was used as inoculum. The results of some of the more important experiments are summarized in Table V.

TABLE V.—*Summary of cross-inoculation studies with Sclerotinia spp.*

Source of culture	Asparagus shoots	Bean pods	Beet roots	Carrot root	Celery stalks	Cucumber fruits	Lemon fruits	Lettuce heads	Parsnip roots	Pea pods	Potato tubers	Sweet potato roots	Turnip roots	Tomato fruits
S. LIBERTIANA														
Bean pods	+[a]	+	(?)	+	+	+	+	+	+	+	–[b]	+	+	+
Carrot roots	+	+	(?)	+	+	+	+	+	+	+	–	+	+	+
Celery stalks	+	+	(?)	+	+	+	+	+	+	+	–	+	+	+
Lemon fruit	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Lettuce heads	+	+	(?)	+	+	+	+	+	+	+	–	+	+	+
Parsnip roots	+	+	–	+	+	+	+	+	+	+	–	+	+	+
Pea pods	+	+	–	+	+	+	+	+	+	+	–	+	+	+
Potato stem	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Salsify roots	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Strawberry fruit	+	+	–	+	+	+	+	+	+	+	–	+	+	+
Sweet potato roots	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Turnip roots	+	+	(?)	+	+	+	+	+	+	+	–	+	+	+
S. INTERMEDIA														
Salsify root	+	+	+	+	+	+	+	+	+	+	–	+	+	+
S. MINOR														
Lettuce heads	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Sunflower plant	+	+	+	+	+	+	+	+	+	+	–	+	+	+
S. RICINI														
Ricinus plants	+	+	(?)	+	(?)	+	+	+	+	+	–	+	+	+

[a] +=result positive. [b] –=result negative.

Twelve strains of the *S. libertiana* type which were isolated from different hosts were used, together with one strain each of *S. intermedia* and *S. ricini* and two strains of *S. minor*. These cultures were used to inoculate nine different hosts that are commonly affected with Sclerotinia decay. In addition, potato tubers, beet roots, and tomato and lemon fruits were inoculated, because the two former commodities do not seem ever to have been found on the market affected with Sclerotinia decay, while the two latter fruits were selected because of their relatively high acid content.

As shown in Table V, practically all of the strains obtained from the various host plants are pathogenic to those hosts commonly found attacked by Sclerotinia. Failure to get infection of the Irish potato was consistent. Both young and old tubers of several different varieties have been inoculated, but no tuber has ever shown even the slightest decay that could be ascribed to Sclerotinia infection. A culture isolated from potato plants in the field also proved negative to the tubers. These results are contrary to those obtained by Bisby (*5*), who inoculated potato tubers with a strain of Sclero-

tinia found parasitizing sunflowers in Manitoba and obtained positive results. The results obtained in the present experiments, nevertheless, indicate that the potato tuber is highly resistant to infection by Sclerotinia. This is in entire agreement with experiences in the market. Up to the present time not a single tuber has been found affected with Sclerotinia rot.

A stalk rot and wilt of potato plants caused by *Sclerotinia sclerotiorum* has been studied and described in Europe for several years. Pethybridge (*22*) observes that although the plants may be seriously affected in certain seasons, the tubers are not decayed. He made mention of the supposition that the fungus could not grow at all on potato tubers, but in 1909 and 1910 he obtained growth and development of sclerotia on slices of living tubers when he inoculated them with a pure culture of *S. sclerotiorum* obtained from ascospores.

In America, so far, the Sclerotinia disease of potato plants is of minor importance. The writer has worked with only two cultures that were isolated from potato plants.[7] One of these was isolated from Florida plants, and the other from potatoes affected with a stalk decay in New Brunswick, Canada. Both of these cultures appear to be *S. libertiana*. In 1921, Lachaine (*19*) found 10 per cent of the potatoes in a field in New Brunswick affected with a stem rot and wilt. The causal organism was found to be a sclerotia-forming fungus which he was inclined to call *S. libertiana*. The crustlike formation of sclerotia on carrot, and the small sclerotia formed on potato agar as shown in his photographs, however, are very similar to those produced by *S. minor*.

The inoculation of beets was very largely unsuccessful or doubtfully positive, in many cases. No marked amount of decay was obtained with any strain, but in some cases the brownish discoloration and decay entered deep enough so that the fungus could be reisolated from the inner tissues. When the reisolations were successful the inoculation is marked positive in the table, but when only a slight discoloration and surface growth of the fungus was evident the experiment is marked with a question mark.

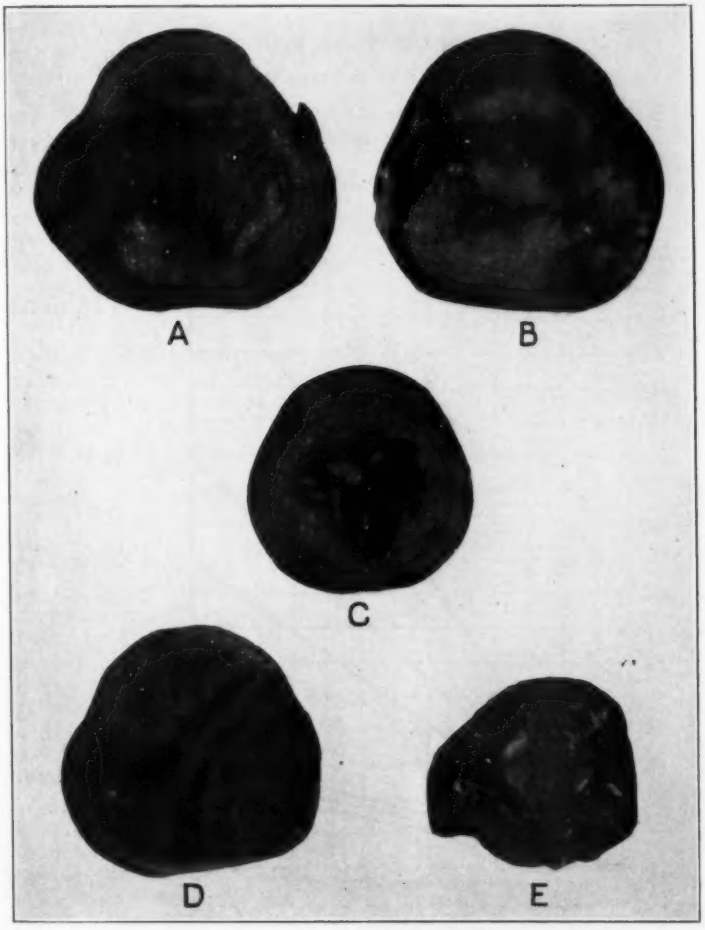
It is readily apparent, in so far as the cross-inoculation studies herein listed are concerned, that the host range of *Sclerotinia* spp. is so wide that it is impossible to separate the different species on the basis of host infection. On the hosts tried—*S. libertiana*, *S. minor*,[8] *S. ricini*,[9] and *S. intermedia*—all produced definite infection and decay, as shown in Table V. On most host plants the type of decay produced by the above species is quite similar and it is not until peculiarities of mycelial growth become evident or sclerotial formation takes place that the species can be distinguished (pl. 5 A, B, C, D, and E).

While there were slight variations from time to time between the growth and appearance of the *S. libertiana* strains obtained from different hosts, there were no more differences than those occasioned by fluctuations in temperature and humidity when the same strain was used in separate experiments. All observations and experiments conducted thus far indicate that all of the large sclerotial forms under observation belong to the same species. With the exception of

[7] Potato cultures were obtained through courtesy of H. H. Whetzel, of Cornell University, Ithaca, N. Y.

[8] Cultures from lettuce by Ivan C. Jagger and W. S. Beach; from sunflower, by C. E. Owens; and from yarrow, by H. A. Edson.

[9] Culture by G. H. Godfrey.



Slices of cucumber inoculated with four species of Sclerotinia, showing growth of the fungi, and the type of decay produced at room temperature

A.—Decay produced by *S. libertiana*
B.—Decay produced by *S. intermedia*
C.—Control
D.—Decay produced by *S. ricini*. Note heavy brown growth of conidia stage
E.—Decay produced by *S. minor*, showing more rapid and complete decay of the cucumber and formation of sclerotia

S. ricini, which produces a darker-colored decay than that of the other species, there is not enough constant difference in texture and color of the lesions to justify any attempt to differentiate between the different decays produced by the different species.

TEMPERATURE STUDIES

GROWTH ON CULTURE MEDIA

In cultural studies of *Sclerotinia libertiana*, *S. minor*, and *S. intermedia*, the greatest differences in habit of growth have been found

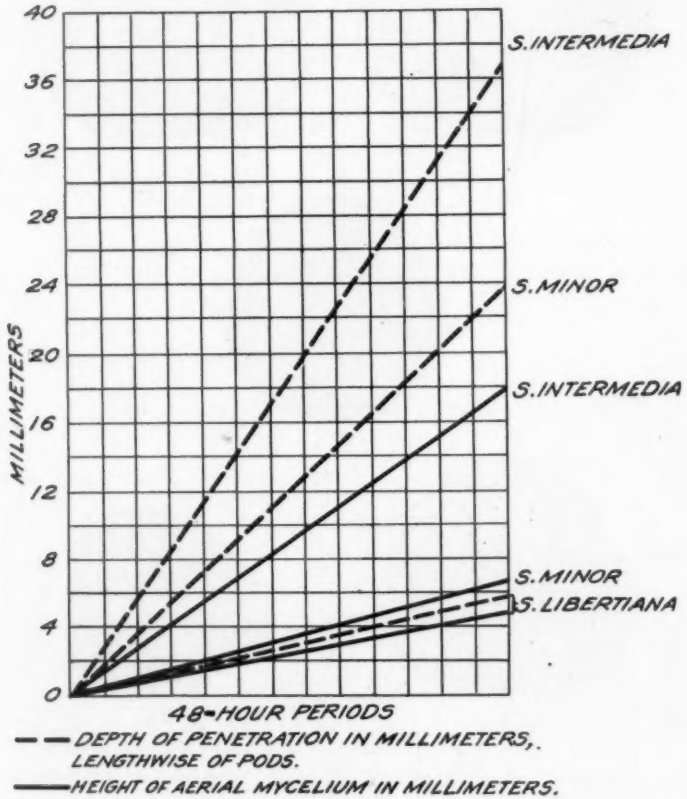


FIG. 1.—Graph showing average radial growth in millimeters of 14 strains of *S. libertiana*, 4 strains of *S. minor*, and 1 strain of *S. intermedia* on potato-dextrose agar at 0° C. during 10-day period

when low temperatures are involved. The mycelium of each species has been found to remain viable after exposure to −5° C., and to make growth on potato-dextrose agar below 0°, but the rates of growth are widely different. This is especially noticeable with *S. intermedia*, which, as a rule, grows about twice as fast as either of the other species. Figure 1 shows the results of an experiment

involving 14 strains of *S. libertiana*, 4 of *S. minor*, and 1 of *S. intermedia*. The cultures were made on potato-dextrose agar. The inoculum in each instance was a small bit of agar containing a young, vigorously growing mycelium. All cultures were held at 15° C. for 12 hours, in order to let the fungi establish themselves, and were then placed in cold storage at 0°. The temperature was carefully checked throughout the experiment, and the range was found to be −0.5 to 0°, as shown by Figure 2.

During the 12-hour period in which the cultures were all at 15° C., *S. minor* and *S. intermedia* made a radial growth of 0.5 mm., while *S. libertiana* grew 1 mm. It will be noted that immediately following storage at 0° the rate of growth of *S. libertiana* was considerably checked. *S. minor* continued at a slightly faster rate than *S. libertiana*, while *S. intermedia* made rapid growth in comparison. At the end of the 10-day period, *S. intermedia* had made slightly more than twice as great a radial growth as either of the other species This ratio of growth was maintained up to temperatures slightly above 0°. Thereafter these differences become gradually less marked until at 7° each of these species makes approximately the same rate of growth (pl. 6, C). At all temperatures above 7°, *S. libertiana* gradually makes more rapid headway until when a temperature of 20° is obtained, it grows about twice as fast as either of the other species (pl. 6, A, B, and D).

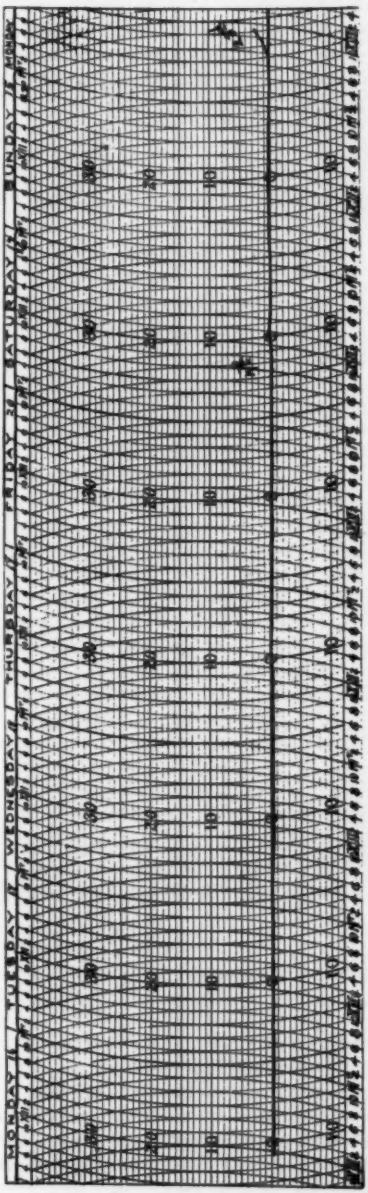


FIG. 2.—Thermograph record showing temperature maintained in 0° C. chamber during experiment to determine rate of growth of Sclerotinia species on potato-dextrose agar

Ascospores germinate very readily in sterile water and nutrient broths throughout a wide range of temperatures (pl. 3 C, *a*, *b*, *c*, *d*). Successful germination has been obtained with the strains of *S. libertiana* tested at temperatures as low as 3 to 4° C. (pl. 3, D, *a* to *g*) and as high as 30 to 31°. Ascospores on the surface of a nutrient agar plate, however, failed to germinate when subjected to a temperature of 30 to 31° for a week. This indicates that the spores can not withstand the drying effect of this high temperature unless they are surrounded with moisture in great abundance.

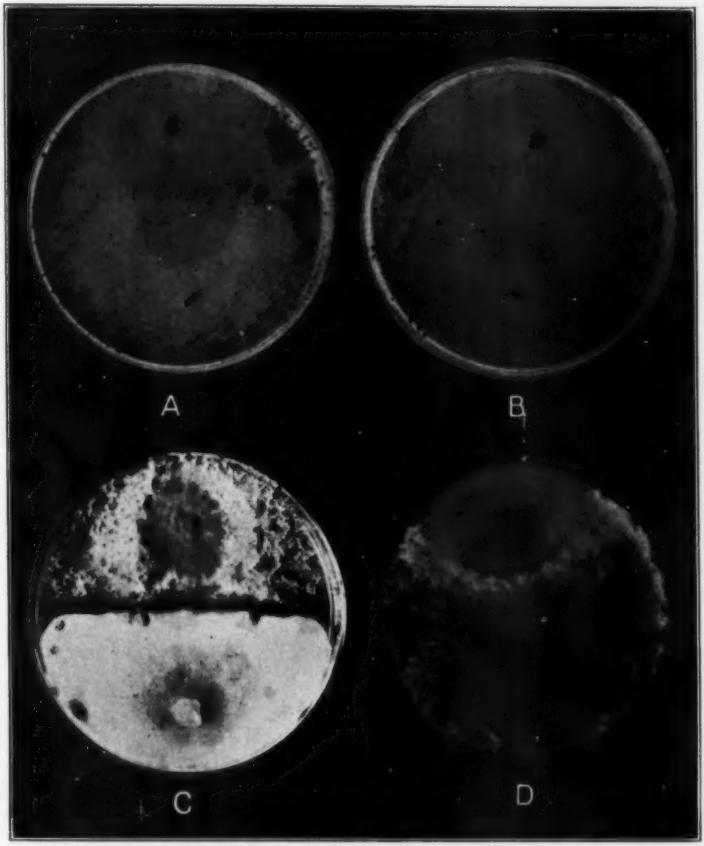
Although no temperature experiments were made with ascospores used as inoculum for infecting plants, it was of interest to the writer to find that the ascospores could withstand such low temperature. "Shooting" ascospores of *S. libertiana*, caught upon a potato-dextrose agar plate which was placed immediately in a refrigerator, retained their vitality after being subjected to a temperature that fluctuated between 5° and −3° C. for 4½ days. No growth was made the first day, and only slight growth was evident within the 4½-day period. However, after the plate was placed at room temperature for 3 days, the surface of the medium was completely covered with the fine, white, cottony mycelium characteristic of Sclerotinia.

GROWTH IN HOST TISSUES

From a market point of view, it is of extreme importance to determine the temperature relations of Sclerotinia, and to determine whether there is any possibility of controlling decay by low temperatures. So far as the writer has been able to ascertain, the lower temperature limits of infection by Sclerotinia have never been determined. In order to obtain information along this line, several inoculation experiments have been carried out in which various hosts were tested.

Figure 3 presents in graph form the results of a typical experiment. Fresh bean pods of the Golden Wax variety were sterilized in mercuric chloride (1 to 1,000) for three minutes, and thoroughly washed in sterile distilled water; each pod was then cut in half and placed in a large test tube containing 3 c.c. of sterile water. The freshly cut ends of these pods were inoculated with young mycelium from potato-dextrose agar plate cultures. After inoculation all cultures were placed immediately in cold storage at 0°. At the end of 26 days the cultures were taken out and examined, both for penetration of the host tissues and aerial growth of the fungus. In each case it was found that the depth of penetration of the fungus was somewhat greater than the corresponding height of the aerial growth. *S. libertiana* penetrated along the long axis of the pod a distance of 6 mm., while *S. minor* and *S. intermedia* penetrated 24 mm. and 37 mm., respectively. The rate of growth as here shown is somewhat slower than that of the same fungi on potato-dextrose agar. However, the same relative positions are held as regards differences in growth rate among the three species.

This experiment taken together with others of similar nature, seems to show conclusively that it is possible for vegetables to become infected with Sclerotinia and for a slow decay to result, even though the product is held at 0° C. In other experiments in which beans, peas, and carrots were used in inoculations at 3° to 4°, decay took



Cultures of *Sclerotinia libertiana*, *S. intermedia*, and *S. minor* on potato-dextrose agar plates, showing differences in rate of growth at 7° C. and 20°

A.—*S. intermedia* (above) and *S. minor* (below), planted together, and held at 20°. *S. intermedia* did not form any sclerotia, and its growth was slow in comparison with *S. minor*

B.—Two strains of *S. intermedia*, planted together, and held at 20°. The strain from salsify (above), and the strain from carrot (below), grew at equal rates and met in the center of the plate, each forming sclerotia

C.—*S. intermedia* (above) and *S. libertiana* (below), grown together, at 7°. At this temperature each species grows at about the same rate

D.—*S. intermedia* (above), and *S. libertiana* (below), showing the latter growing about twice as fast as *S. intermedia* at 20°

place much more readily, but developed to no marked extent until after the products had been held for about two weeks. In every case *S. intermedia* grew fastest and produced most decay at the temperatures just mentioned.

Jagger (*18*) found, in his studies of *S. minor* on lettuce, that this species produced a more rapid decay than *S. libertiana* and that the mycelium was less conspicuous in the decaying plants. In the writer's experience these observations hold true for most of the hosts

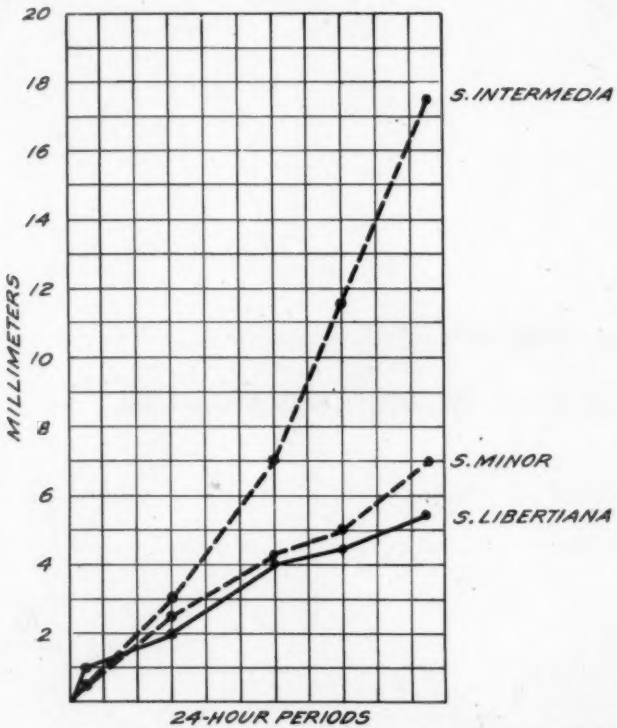
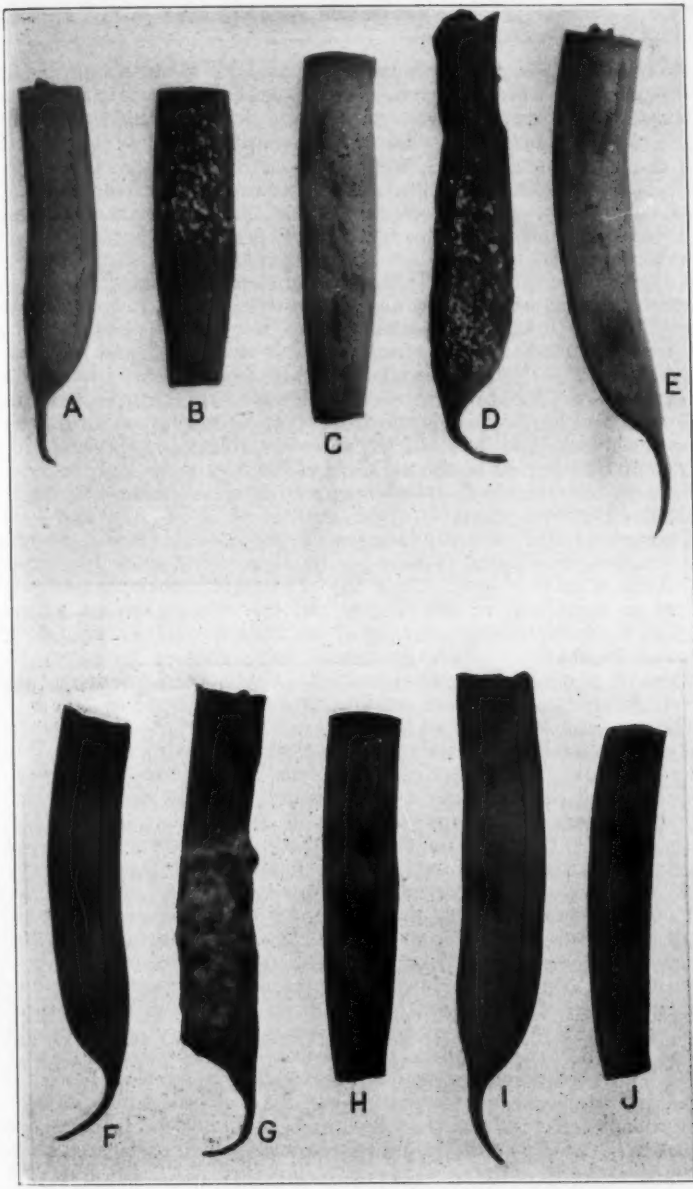


FIG. 3.—Graph showing growth of three species of Sclerotinia on bean pods when held at 0° C. for 26 days

on which cross-inoculation studies have been made, with a few exceptions, which might be explained by temperature influences or variations in host susceptibility. In an experiment in which fresh bean pods were inoculated on freshly cut surfaces and placed in test tubes containing 3 c. c. of sterile water and held at 20° C., the pods which were inoculated with *S. minor* decayed faster than those inoculated with either *S. libertiana* or *S. intermedia*. Parallel inoculations of the above experiment were held at 7°, and, as shown in Plate 7, A, E, F, J, very good control was accomplished during this 10-day experiment.



Fresh bean pods inoculated with *Sclerotinia libertiana*, *S. minor*, and *S. intermedia*, showing growth of these fungi and decay produced at 20° C. as compared with that produced at 7° during a 10-day period

A, B.—Development of a strain of *S. minor* from lettuce, at the temperatures indicated (A=7° and B=20°)

C.—Control

D, E.—A strain of *S. minor* from sunflower. Note complete decay and the formation of sclerotia at 20°, and the absence of decay at 7°

F, G.—*S. libertiana* growth at the indicated temperatures. At 20° decay is not as rapid and complete as that produced by *S. minor*. At 7° the fungus is just beginning to make headway within the 10-day period

H.—Control

I, J.—Slow decay produced by *S. intermedia* at 20°, and a slightly more rapid decay at 7°, as compared with *S. minor* and *S. libertiana* at the same temperature

At temperatures ranging between 27° and 30° C. a large proportion of inoculations were negative. While parallel agar cultures carried at these temperatures showed growth and development, the progress made was comparatively slow and the appearance of the culture was more or less abnormal, the growth of *S. intermedia* being more inhibited than either *S. minor* or *S. libertiana*. With the exception of *S. intermedia*, which stops growing at 30° to 31°, each of these cultures has shown ability to make slight growth on potato-dextrose agar at 32° to 33°.

To determine the ability of these fungi to produce infection at high temperatures, freshly cut young carrot roots were inoculated and placed in a high temperature chamber. For the nine-day period of the experiment the temperature varied between 31° and 34°, with a mean of 32°. No growth was found in any culture. After these cultures were held for five days at room temperature there was no evidence of life. Control cultures run at room temperature showed vigorous growth and decay of the carrots within a few days, so there can be no question as to the viability of the organisms. Apparently, these fungi are unable to grow enough to establish themselves in the tissues of the host plants at a temperature of 32°.

The amount of Sclerotinia decay found under transit, storage, and market conditions is necessarily dependent upon the extent of infection in the field. Once the product is contaminated with spores or mycelium of the fungus and has started on its way to market, the most effective method of controlling the amount of decay is by refrigeration. As experiments have shown, however, this method of control is somewhat limited. Under the present methods of refrigeration a car which maintains a temperature of 40° F. at the bottom and 45° to 46° at the top is considered a good refrigerator, but unfortunately Sclerotinia will develop and cause decay at these temperatures. The writer's experiments show that *S. libertiana*, *S. minor*, and *S. intermedia* can infect freshly made wounds in suitable host plants and cause appreciable decay at these temperatures. In fact, the experiments conducted in cold storage show quite clearly that infection of freshly wounded tissues can take place even at 32° F. It is therefore manifestly impossible to get absolute control of these fungi by any practical method of refrigeration which would not also be disastrous to the produce. From a practical viewpoint, however, the experiments show that although incipient infections or contaminated wounds present at shipping time may develop decay at a temperature of 40° to 45° F., little spreading from one plant to another or development of decay in unwounded tissues is to be expected during the ordinary transit period.

The most effective method of preventing Sclerotinia decay in transit is, of course, to prevent the loading of infected produce or badly wounded produce that has had a chance to become contaminated. Clean, carefully graded vegetables will carry well under present methods of refrigeration at temperatures of 40° to 45° F. The precooling of vegetable produce is advisable whenever possible, however, on account of the fact that without precooling it takes from one to three days in the refrigerator car to reduce the temperature of the product to a point which is really effective in controlling Sclerotinia decay. Small lesions or contaminated wounds present

at shipping time in vegetables which are not precooled may therefore develop considerable decay during the first few days of transit in spite of the fact that the car may show a perfect icing record and a temperature of 40° to 45° F. on arrival at market.

It is rather difficult to control Sclerotinia decay in the field on account of the fact that the soil soon becomes heavily seeded with sclerotia if the same crop is planted year after year, and it is often difficult to find profitable truck crops to rotate which are not also subject to attack by this fungus. The soil may be sterilized by means of heat or chemicals, but this is practicable for only small plots and greenhouses. The selection of the more upright varieties of lettuce and the practice of general field sanitation, together with two or three year crop rotation system, seems to be the best recommendation for the control of Sclerotinia diseases in the field.

In summarizing all studies and experiments made upon the various Sclerotinia cultures isolated from vegetable produce on the market, with the exception of *S. intermedia*, which was discovered during the progress of these investigations, and one other undetermined strain, all cultures have shown morphological as well as physiological characteristics of *Sclerotinia libertiana* Fckl. More than 90 per cent of all Sclerotinia cultures isolated by the writer in the last four years belong to this species.

SUMMARY AND CONCLUSIONS

Sclerotinia libertiana Fckl. and related species cause great damage to truck crops during transit and storage, as well as in the field.

All strains of Sclerotinia under observation produce a soft, watery, odorless type of decay of the host plants.

Observations on the market indicate that produce harvested during wet weather is especially susceptible to Sclerotinia infection and decay during transit.

Vegetable produce having little or no visible decay at time of shipment may show a high percentage of infection and decay upon arrival at market.

S. libertiana is not limited to vegetable crops, but may also attack fruits. The manner of growth of the host and the opportunities for infection seem to be the important limiting factors.

From 20 to 50 per cent of the cars of carrots, lettuce, and celery which the Bureau of Agricultural Economics, United States Department of Agriculture, is called upon to inspect show decay caused by Sclerotinia. The amount of infection often averages higher than 50 per cent, and in some instances 100 per cent of the shipment is affected.

Morphological and cultural studies show that there is no difference between cultures arising from sclerotial and mycelial plantings, other than variations in rate of growth.

Cultural studies on agar indicate that at temperatures approaching the minimum for the strain, the sclerotia formed are likely to be larger than normal for room temperature. At temperatures nearing the maximum for the fungus the sclerotia formed are often smaller than normal.

On media favorable for the development of the fungus the sclerotia formed are larger than those formed on unfavorable media.

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No definite period of dormancy is necessary to prepare sclerotia for production of apothecia. The ecological factors of moisture, temperature, and light determine apothecial formation.

Small sclerotia are especially susceptible to decay if kept too moist. This is one reason why it is usually much more difficult to obtain apothecia from *S. minor* and *S. intermedia* than from *S. libertiana*.

Morphological studies, including measurements of sclerotial apothecia, asci, and spores, show that all of the large sclerotia, forms found upon the market belong to the species *S. libertiana*.

All strains of Sclerotinia under observation produced microconidia to a greater or less extent sometime during their life history.

The amount of available food is the chief factor in determining the time of microconidial production. Media unfavorable for the vegetative growth of the fungus induces a rapid and prolific production of microconidia.

Previous to this time, as far as the writer knows, no one has ever observed germination of the microconidia in the genus Sclerotinia. In these studies the writer has been able to induce the microconidia of several strains to germinate.

The experiments herein described indicate that the microconidia do not play an important rôle in the life history of the genus Sclerotinia.

Artificial infection of host plants is readily obtained by inoculation with sclerotia and mycelium, under favorable conditions. Moisture and temperature are the important limiting factors in determining infection. Infection may take place through unwounded as well as wounded surfaces.

The more succulent plants and plant parts are penetrated most rapidly, while the woody tissues are a great deal more resistant, thus indicating that the mechanical make-up of the plant may play a part in resistance to Sclerotinia infection.

Infection of freshly cut surfaces of turnip and carrot roots, by holding them over "shooting" apothecia, has proved successful. An ascospore dilution in distilled water sprayed over lettuce leaves also gave positive results.

Cross-inoculation studies show that *S. libertiana*, *S. intermedia*, *S. ricini*, and *S. minor* are capable of producing decay in a wide range of vegetables.

None of the Sclerotinia cultures were found to be pathogenic to potato tubers. Beet roots were also highly resistant, although a few inoculations produced slight decay.

The host range of the Sclerotinia species considered in this study was so wide that it is impossible to separate them on the basis of host infection. The decay produced (*S. ricini* excepted) is so similar that it is not until peculiarities of mycelium or sclerotial growth become evident that the species can be distinguished.

S. minor produced a more rapid decay than any of the other species when inoculated into bean pods and other host plants and held at 20° C.

Inoculation studies as well as plate cultures show that Sclerotinia is able to grow and produce infection at temperatures as low as −0.5° to 0° C. Of all strains tested, *S. intermedia* grew fastest and produced most decay at this temperature.

With the exception of *S. intermedia*, which stops growth at 30° to 31° C., all other strains have shown ability to make slight growth on potato-dextrose agar at 32° to 33° C. Inoculation experiments conducted at this temperature proved negative.

Ascospores germinate readily in sterile water and nutrient solutions throughout a wide range of temperatures. Successful germination has been obtained at temperatures as low as 3° to 4° C. and as high as 30° to 31°.

Experiments show that incipient lesions and contaminated wounds will develop decay during transit under refrigeration temperatures of 40° to 45° F., but that development of new lesions and spreading of the fungus from one plant to another during the ordinary transit period will be fairly well controlled. Clean, carefully graded vegetable produce which has been precooled at loading point should arrive on the market in good condition if held throughout the trip at temperatures of 40° to 45° F.

A summary of all observations and experiments shows that more than 90 per cent of the Sclerotinia cultures isolated from vegetable produce on the market during the past four years belong to the species *S. libertiana*.

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AGE OF SEEDLINGS AS A FACTOR IN THE RESISTANCE OF MAIZE TO SODIUM CHLORIDE[1]

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INTRODUCTION

Much work has been done in recent years in comparing different genera, species, varieties, and strains of plants as to their resistance to the effect of salt solutions. Harter (*3*),[2] Kearney and Harter (*5*) and others have called attention to the opportunity for selecting and breeding the most resistant strains for alkali soils common in the arid portions of the United States, but apparently little has been accomplished along such lines.

The work of the writers, the results of which are presented in this paper, was taken up in 1923 at the suggestion of G. N. Collins, botanist in charge of biophysical investigations of the Bureau of Plant Industry, United States Department of Agriculture, who suggested the water-culture method, whereby great numbers of individuals could be handled and observed in a short time, as a means of segregating individuals showing marked resistance to the effects of the salts contained in alkali soils.

Since sodium chloride is a predominating salt in the southern Arizona valleys,[3] it was decided to make use of this salt alone in the preliminary experiments (*6*). Corn was chosen as the crop to use because of the ease of handling and transplanting the seedlings and its suitability for genetic studies. Extreme variation in tolerance of seedlings of different sizes to the effects of sodium chloride was encountered soon after the investigations were started, and the results obtained in this phase of the work are presented and discussed in this paper.

EXPERIMENTAL METHODS

The seeds were sprouted on a lime-sawdust germinating table (*4*), and allowed to remain until the radicles had attained convenient length for suspending in the salt solutions, then the seedlings were transferred to perforated aluminum disks which were floated by means of sealed glass buoys on the surface of the solutions contained in enameled milk pans (*1*).[4] These culture pans contained 3 liters of the salt solution, and the volume was kept constant by the use of Mariotte flasks suspended above each pan. In all of the work prior to May 22 the seedlings were assorted according to the length of the primary radicle, and 100 plants of uniform radicle length were placed

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[2] Reference is made by number (italic) to "Literature cited," p. 640.

[3] The results of analyses by the Bureau of Soils, U. S. Department of Agriculture (*6*), indicate that sodium and chlorine comprise on an average 56.3 per cent of the total alkali salts in the soils of the U. S. Field Station at Sacaton, Ariz. (Na 31.9 and Cl 24.4).

[4] The writers gratefully acknowledge the helpful assistance rendered by J. F. Breazeale, associate biochemist in the Office of Western Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture, who performed work along similar lines in 1921 and 1922.

on each disk. It soon became evident that the number of seedlings surviving the salt solution increased somewhat in proportion to the length of the radicles. In the effort to determine the limit of age or size influence on resistance, tests were made with seedlings which had been allowed to develop primary roots 100 to 125 mm. long before subjecting them to the solutions. After finding increased resistance, even at this advanced stage, it became necessary to resort to some other method of classification according to size in order to work with still older seedlings, since at this stage many secondary seminal roots had developed and difficulty was encountered in handling and measuring the roots. It was found that the stage of development of the plumule afforded a very satisfactory means of classing the seedlings at this age, and no measurements were necessary. After May 22 all classification was based on the plumule development, and the radicle length was disregarded.

In the preparation of the seedlings for testing in groups according to stage of plumule development, they were sorted and transferred to culture pans containing tap water as soon as the radicles of the youngest group were of sufficient length to balance the seeds well on the perforated disks. It was usually possible at this time to sort fairly accurately; however, when the desired stage of development was reached they were again classified according to the extent of plumule development and then placed in a salt solution of the desired concentration.

During a greater part of the investigation the seedlings were allowed to remain in the salt solution for 72 hours, but it became evident when working with seedlings in the plumule stage, and especially during warm weather, that this period frequently was too short to bring to the death point more than 50 per cent of the individuals, even in the high concentrations used, and the period was increased to 96 hours.

After subjecting the seedlings to the solutions for the 96-hour period, the number of individuals which apparently had survived in each pan was recorded and the live plants were transferred in groups to other pans containing tap water. Another count was made 72 hours later, and plants which had failed to resume growth of roots or plumule in the water were classed as dead. The extent of the green coloring in the basal portion of the coleoptyle afforded an easy means of distinguishing those seedlings which were likely to overcome their injuries when removed to tap water, and it was seldom that more than 4 or 5 per cent of seedlings of the first selection were later eliminated.

After working with varied concentrations for several months the writers became convinced that the stage of growth of the seedlings was a greater factor in determining the toxic limits of the plants than small differences in the amount of salt in solution. It was therefore decided to investigate this phase of the problem, and a concentration of 22,000 parts per million of sodium chloride was adopted as a standard for further study.

In view of the methods employed in this experiment it isobvious that the results are not comparable with those obtained bymost of the previous investigators, some of whom have sought to fix the maximum tolerance or toxic limits of various plants for alkali salts. In previous investigations effort apparently has been made to deter-

mine the highest concentration of salt to which a plant can be exposed without showing injury to any of its organs or without seriously affecting its ordinary behavior or growth. In this experiment the purpose was to kill off a greater part of the plant population in order to isolate those individuals which showed a marked resistance. It will be seen that salt concentrations used in this experiment are considerably higher than those used by most of the other investigators, who have seldom carried the concentration to the point where the entire plant was killed.

RESISTANCE OF SEEDLINGS WITH DIFFERENT LENGTHS OF RADICLES

At the beginning of the experiment seedlings were used which had developed radicles only 25 to 50 millimeters long with plumules still encased in the coleoptyles. It soon was discovered that seedlings of this size were unsatisfactory for use in the higher concentrations of salt, for, where the percentage of salt was as great as 1.8 per cent, no further elongation of the radicle was observed after a few hours in solution. A few of the seedlings developed a hardened bulbous enlargement just behind the tip of the radicle, and in such cases the root remained turgid and healthy but without further growth of radicle or coleoptyle. On most of the seedlings, however, the radicle became flaccid during the first 24 hours in the salt solution, and at the end of 72 hours many of the primary roots had sloughed off, leaving only a stub attached to the mesocotyl (*2*). At the end of this period the coleoptyles of many of the seedlings had assumed a yellowish color and showed signs of decomposition. A few seedlings however, after losing their radicles began to develop secondary roots from the base of the mesocotyl or laterally from the remaining stub of the radicle. In such cases there occurred a quick response in growth of the plumule, and development was continued though slowly for several days while exposed to the salt solution. Inasmuch as the primary or axial root soon dies under field conditions in the early stages of development of the maize plant, it was decided that the most valuable test of resistance should occur after the brace roots or permanent roots had begun to function.

The percentage of seedlings with radicles of various lengths which survived after 72 hours in the salt solutions are shown in Table I.

TABLE I.—*Percentage of maize seedlings surviving after 72 hours in salt solution, when the primary radicles were at different lengths between 25 and 100 millimeters*

Date	Percentage of sodium chloride	Length of radicle (percentage of seedlings surviving *)					
		25 mm.	38 mm.	42 mm.	51 mm.	88 mm.	100 mm.
1924							
Mar. 29	2.0		11.7±2.33				
Mar. 31	2.2		11.2±2.18				
Apr. 5	2.0	6.9±1.76					
Apr. 6	2.0		6.9±1.76	18.4±2.48			
Do	2.2			14.8±2.33			
Apr. 14	2.2	3.3±1.20	11.2±2.13	11.4±2.45			
Apr. 21	2.2		4.9±1.47		8.6±2.00		
May 22	2.2	3.1±1.160	8.9±2.04		12.8±2.35	22.8±2.84	23.1±2.84

* The irregular growth of the radicles made it impracticable to select a sufficient number of seedlings of the various radicle lengths to represent each group for comparison on the same day.

Since it was found impracticable, on account of fluctuating weather conditions, to obtain consistent results from seedlings selected on different dates, it is suggested that the data in Tables I, II, and III be studied and compared under one date at a time.

The heaviest mortality occurred where the radicles were only 25 millimeters long. In this stage the cotyledon was just pushing through the seed coat and only the primary root had emerged. The greatest number of plants survived from stages where the radicles had attained considerable length and where the plumule had ruptured the tip of the coleoptyle.

In testing the effect of different concentrations on the seedlings it was noted that a more rapid plumule growth often developed in concentrations of 18,000 to 20,000 parts per million than in weaker solutions. It was discovered that this could be attributed to the rapid decay of the primary radicle and the prompt production of permanent roots to carry on the development. In order to test the rapidity of response to the destruction of the primary radicle, several comparisons were made between pan cultures of seedlings in part of which the radicles had been excised with a scalpel about 6 millimeters from the base of the mesocotyl and others which received no mutilation. The percentage of seedlings remaining alive after 72 hours in the salt solutions is given in Table II.

TABLE II.—*Percentage of maize seedlings with excised and normal radicles surviving after 72 hours in salt solution (22,000 parts per million)*

Date	Percentage of seedlings surviving	
	Radicles excised [a]	Radicles not excised
1924		
Apr. 6	15.0±2.41	2.5±1.05
Apr. 14	40.8±3.37	14.8±2.41
Apr. 21	21.0±2.75	5.7±1.62

[a] Seedlings having radicles approximately 40 millimeters long were used, and approximately 100 plants of each treatment were used on the respective dates.

The results in Table II seem to indicate that any form of injury to the primary radicles of the seedling stimulates the early production of permanent roots, and these latter appear to enable the seedling better to withstand salt solutions than do the primary roots.

The results also indicate the necessity of strictly standardizing any method of testing seedlings for alkali resistance. It is obvious that any injuries to the seedling roots in handling might easily influence the number of plants selected as resistant where the growth of the plumule was used as a criterion.

The behavior of the seedlings with excised radicles suggests that the apparent resistance exhibited by some very young seedlings whose primary radicles remained turgid with hardened bulbous tips, as previously described, may be of little significance as their response in growth when transferred to water was not nearly so rapid as the surviving plants with excised radicles.

RESISTANCE OF SEEDLINGS WITH VARIOUS STAGES OF PLUMULE DEVELOPMENT

After finding that the older seedlings could be more easily and accurately classified by the stage of plumule development than by the length of radicles, the seedlings, in all of the later experiments after being sorted and placed on the disks, were allowed to remain in tap water until the youngest group reached the stage when the leaf roll had just emerged from the tip of the coleoptyle, then the disks, each containing 100 plants, were floated on the salt solutions. The percentage of seedlings of different sizes which survived an exposure of 96 hours to the salt solution (22,000 parts per million) is shown in Table III.

TABLE III.—*Comparison of the percentage of seedlings at different stages of plumule development surviving after 96 hours in salt solution (22,000 parts per million)*

Date	Well out of coleoptyle [1]	Just prior to unfurling first leaf	First leaf unfurled	Second leaf appearing
1924				
May 31	15.2±2.67	53.5±3.11		7.0±2.35
Do	19.0±2.65			
Do	7.0±1.72			
June 3	7.2±1.72	61.0±3.29		
Do	20.0±2.69	44.0±3.35		
June 9		17.3±2.53	10.0±2.02	9.4±1.99
June 15		26.0±2.96	10.0±2.02	3.0±1.25
Do		21.0±2.75		
June 25	10.9±2.11	25.3±2.92		6.4±1.63
Do	20.0±2.69			
Do	35.0±3.22			
July 1		42.2±3.23	15.3±2.59	12.3±2.44
Do		32.5±3.32		
July 6		32.0±3.14		8.0±1.83
Do		25.0±2.92		
July 22	16.7±2.53	31.0±3.12	5.5±1.82	9.4±2.01
Do		54.5±3.07		

[1] The radicles at this stage were from 60 to 100 millimeters long. The other three groups represent a more advanced stage of growth than classification shown in Table II.

From the data in Table III it is apparent that there is a critical stage in the development of the seedlings at which their metabolic functions are less interrupted by toxic salts than at any other time. This stage occurs about the time when the first leaf has pushed out of the coleoptyle for 10 or 15 millimeters, and but a few hours before it begins to unfold into blade form. An explanation for this might be found by determining the rate of reduction of the stored food in the endosperm, and the stage at which the seedling begins to decline in vigor when not provided with nutrient material from an outside source.

While the data shown in Table III are far from consistent, especially when comparing results obtained from series of plants handled on different dates, they seem, however, to indicate clearly that the stage of development is of primary importance in determining the relative resistance of seedling to toxic salt solutions, and that the most resistant stage to sodium chloride is just prior to the unfolding of the first leaf (fig. 1, stage No. 2). When the data are studied under one date at a time, it will be observed that there are no discrepancies; all the results indicate an increasing resistance as seedlings develop after first sprouting, until the leaves begin to unfold, and then a diminishing resistance as they become older.

WEATHER CONDITIONS A FACTOR IN ALKALI TOLERANCE

The wide fluctuations in the percentage of survivors on different dates were apparently due to changes in weather conditions, while the seedlings were exposed to the salt solutions. No detailed notes were taken on the daily behavior and appearance of the seedlings in relation to weather conditions, but it was noted when making the counts that the number of survivors was likely to be small if exceptionally high temperature in combination with high wind velocity and low relative humidity prevailed during a considerable part of the time when the seedlings were being tested. It was also observed that when cloudy weather, with high relative humidity prevailed during one or more days of the test, the number of surviving seedlings usually increased.

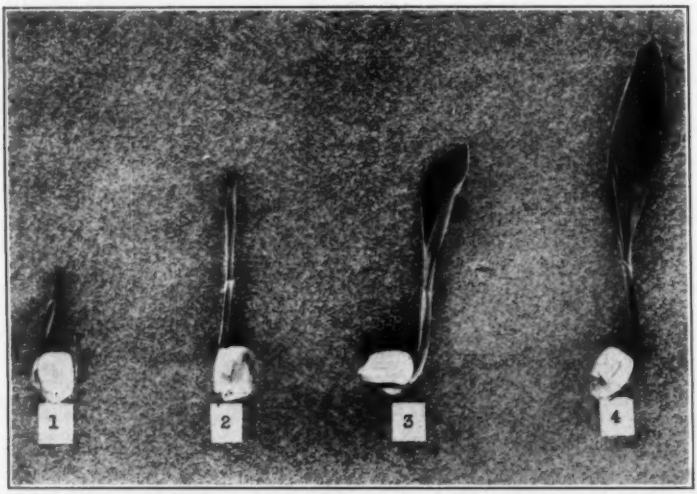

FIG. 1.—The four stages of plumule development at which maize seedlings were subjected to concentrated sodium-chloride solutions. Stage No. 2 illustrates that of greatest resistance, and stage No. 4 that of least resistance

The mean maximum and minimum temperatures, relative humidity, and wind velocity, during the three and four day periods while the seedlings were being tested are given in Table IV.

The data in Tables III and IV indicate that the high relative humidity occurring during the period May 28 to June 3 apparently affected an increase in the seedlings which were counted on May 31 and June 3. During June 6 to 9 there occurred a period of low relative humidity accompanied by unusually high wind velocity and its effect is reflected in the relatively small percentage of survivors recorded on June 9.

There were indications also that seasonal changes influenced the resistance of the seedlings to the salt solutions, and some adjustments were made in the method in order to meet these conditions. It was found that the plants developed more rapidly and showed distress more quickly during the hot months of summer than during the spring and autumn.

TABLE IV.—*Mean maximum and minimum temperatures, relative humidity, wind velocity, and total precipitation, during the three and four day periods while seedlings were exposed to sodium-chloride solutions*

Period	Mean maximum temperature	Mean minimum temperature	Mean relative humidity	Mean wind velocity	Rainfall during period
Mar. 27 to 29	66	43	54	2.4	0.44
Mar. 29 to 31	66	41	58	1.9	
Apr. 3 to 5	79	46	48	2.1	.03
Apr. 4 to 6	80	50	64	3.6	.13
Apr. 12 to 14	78	51	60	2.6	
Apr. 19 to 21	80	44	33	2.1	
May 20 to 22	98	58	31	2.1	
May 28 to 31	91	61	49	2.9	.51
May 31 to June 3	98	61	51	1.8	
June 6 to 9	98	56	29	3.7	
June 12 to 15	109	70	38	1.9	
June 22 to 25	106	67	42	1.6	
June 28 to July 1	106	79	45	2.0	
July 3 to 6	101	75	56	2.2	.04
July 19-22	101	62	40	1.8	

SUMMARY

The use of water cultures in breeding alkali-resistant strains of maize, by selecting individual seedlings which survived strong salt solutions, was studied and various modifications were tested.

The stage of development of the seedlings was found to be an important factor in determining the amount of salt in solution which they were able to withstand.

In testing the youngest stages of seedling development, using various lengths of radicles as a basis of grouping, the resistance increased up to certain limits in proportion to the length of the radicles. The greatest number were killed by strong salt solutions when the radicles were 25 millimeters long, and the smallest number when the radicles were 100 millimeters long.

The extent of the development of the plumule was found to be the most satisfactory indicator for preparing uniform groups of seedlings in the older stages of growth.

The stage at which the older seedlings were most resistant to sodium chloride was just prior to the unfolding of the first true leaf.

Young seedlings with short radicles and undeveloped plumules were found to be most susceptible; the oldest plants with two seed leaves were the next in susceptibility; and the intermediate stages were the most resistant.

Seedlings whose radicles were destroyed earliest by the salt solution made the most rapid plumule growth, due to the rapid development and early functioning of the permanent roots.

Artificial excision of the radicles increased the resistance of the seedlings to a marked degree, causing a prompt and comparatively rapid development of permanent roots and plumule, even while exposed to a strong salt solution.

It was found impossible to obtain consistent results when comparing the behavior of seedlings subjected to salt solutions on different dates, the resistance of the seedlings apparently being influenced profoundly by changes in weather conditions.

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ORGANIZATION OF THE TELIAL SORUS IN THE PINE RUST, GALLOWAYA PINICOLA ARTH[1]

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INTRODUCTION

The short-cycled pine rust now commonly referred to as Galloway a (*2*)[2] was first reported under the name *Coleosporium pini* by Galloway (*9*), who later (*10*) described its effects on the host *Pinus virginiana*. Although he was not fully aware of the exact nature of the germination of the teleutospores, he figured in some detail various stages in the development of the elements of the sorus and brought out some of the most characteristic features of the fungus. The writer was enabled to make a further study of the fungus from material furnished by W. W. Diehl, who collected quantities of the rust for him in the vicinity of Washington, D. C. It will be shown that there is formed a distinct and persistent peridial buffer structure which functions in rupturing the leaf tissues overlying the young sorus and that, following cell fusions, teleutospores are borne in chains. The spores are not sessile in the sense that only one spore is cut off from a basal cell as in Coleosporium. Neither does the basal cell bud to form the spores as in Puccinia.

THE GAMETOPHYTIC ELEMENTS

As no one had questioned the results of Galloway's infection work in demonstrating that the rust is short-cycled, it was to be expected that the cells of the mycelium in the pine leaf would be uninucleated. This is clearly the case. The hyphae are readily stained, although the waxy nature of the young sorus renders it difficult always to get good fixation of later stages. Each cell contains a single nucleus (fig. 1, B).

Vestigial spermogonia are to be found occasionally, but the host tissues above them (pl. 1, A) are not fully ruptured and spermatia are seldom formed. The few spermogonia seen in the sections were located beneath stomata. This may be of no significance, as they are rather broad and the stomata are scattered along parallel lines on the flat side of the leaf. The weft of hyphae just below the base of the primordium is a little denser than that which is to produce a telium. The end cells which are somewhat enlarged send out smaller branches which converge slightly so that they appear to be directed toward the stomatal opening. Galloway's Figure 10 (*10*) might very well have been drawn from a section of a spermogonium.

[1] Received for publication Nov. 13, 1924; issued December, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 651.

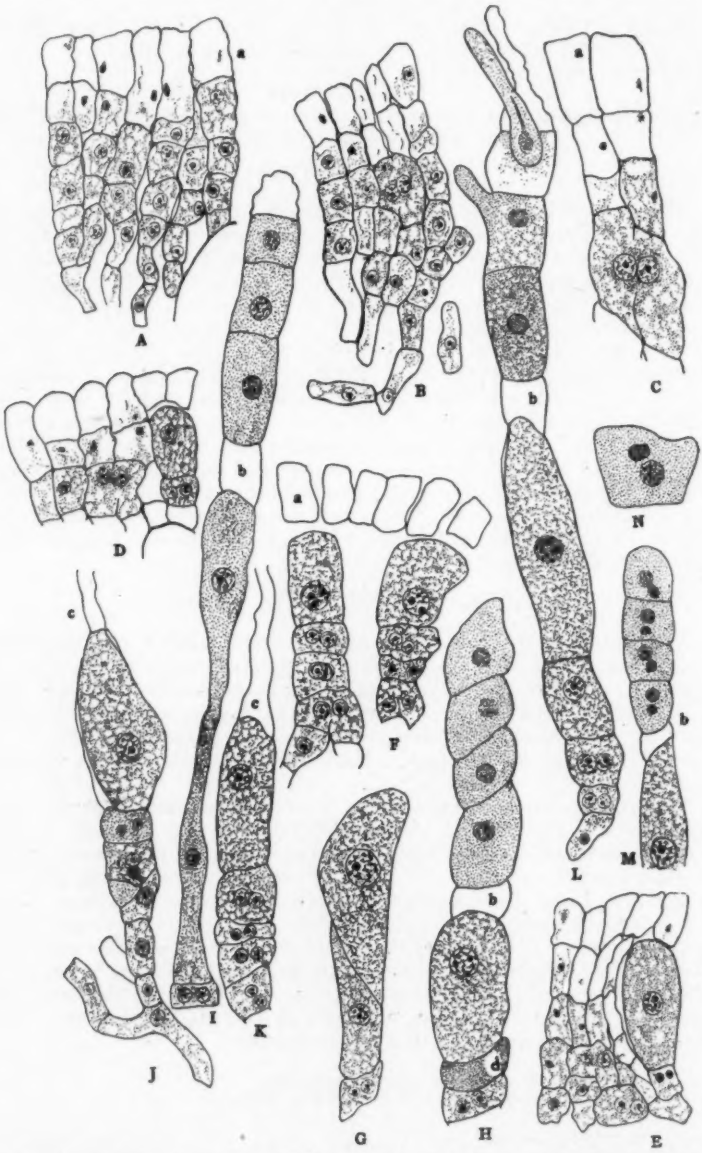


FIG. 1.—A, young primordium before cell fusions; B, first cell fusions, two sterile cells above; C, cell fusion, three sterile cells above; D, first mature spore; E, margin of sorus, fusions at different levels; F, chains of young spores, "peridium" (*a*) above; G, oblique cell divisions; H, I, basidia supported by elongated part of cell wall (*b*); J, K, spore chains in old sorus, stalklike remains of spore above; L, typical spore chain; M, N, basidial cells with additional body in each near nucleus. (C, L, and N are more highly magnified, and M and E less highly magnified, than the others.)

THE PERIDIAL BUFFER TISSUE

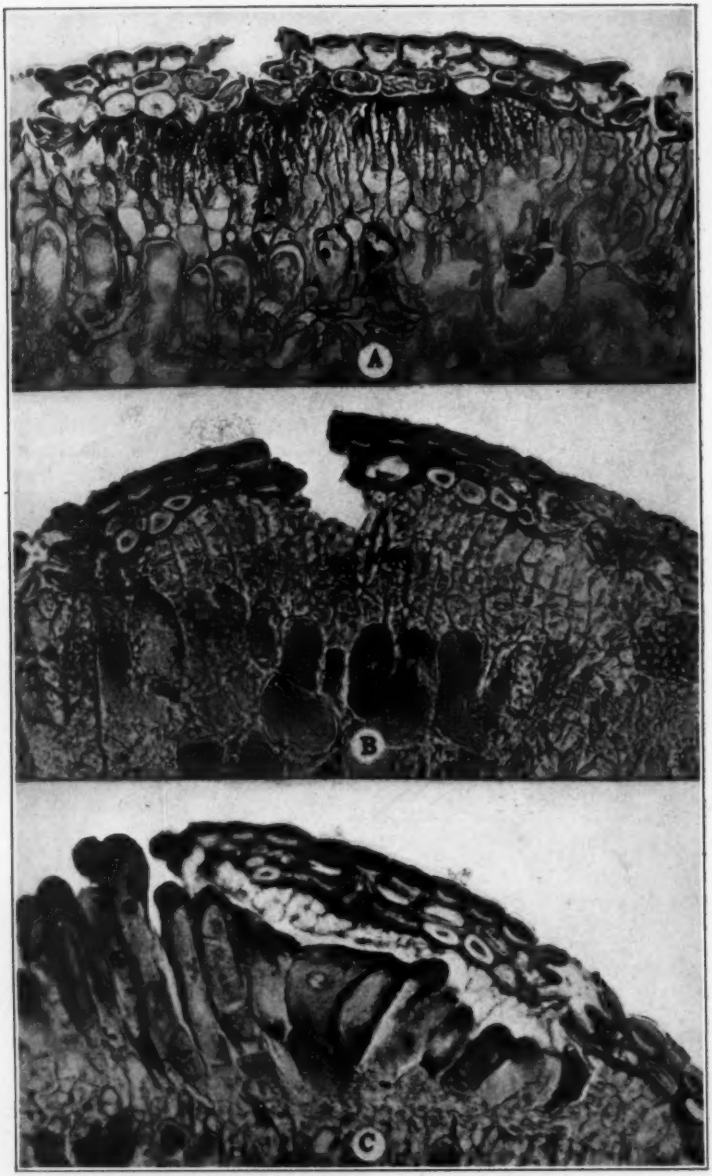
There is very little intermingling or intertwining of hyphae as a preliminary to the formation of the telial sorus. The hyphal complex below is usually no denser than it is in other parts of the leaf. The hyphae push out between the mesophyll cells and widen suddenly just before reaching the ends of the large lobes of these cells. Since the hyphal branches grow straight out against the hypodermal tissue in a solid rank, the epidermis and underlying tissue are stretched and pushed outward. Each chain in the palisade of fungus tissue is composed of four or five uninucleated cells (fig. 1, A). The buffer effect is considerably increased as the terminal cells lose their granular cytoplasmic contents and elongate (fig. 1, C, *a*). The propriety of calling the layer of terminal cells which persists a peridium is discussed later.

CELL FUSIONS

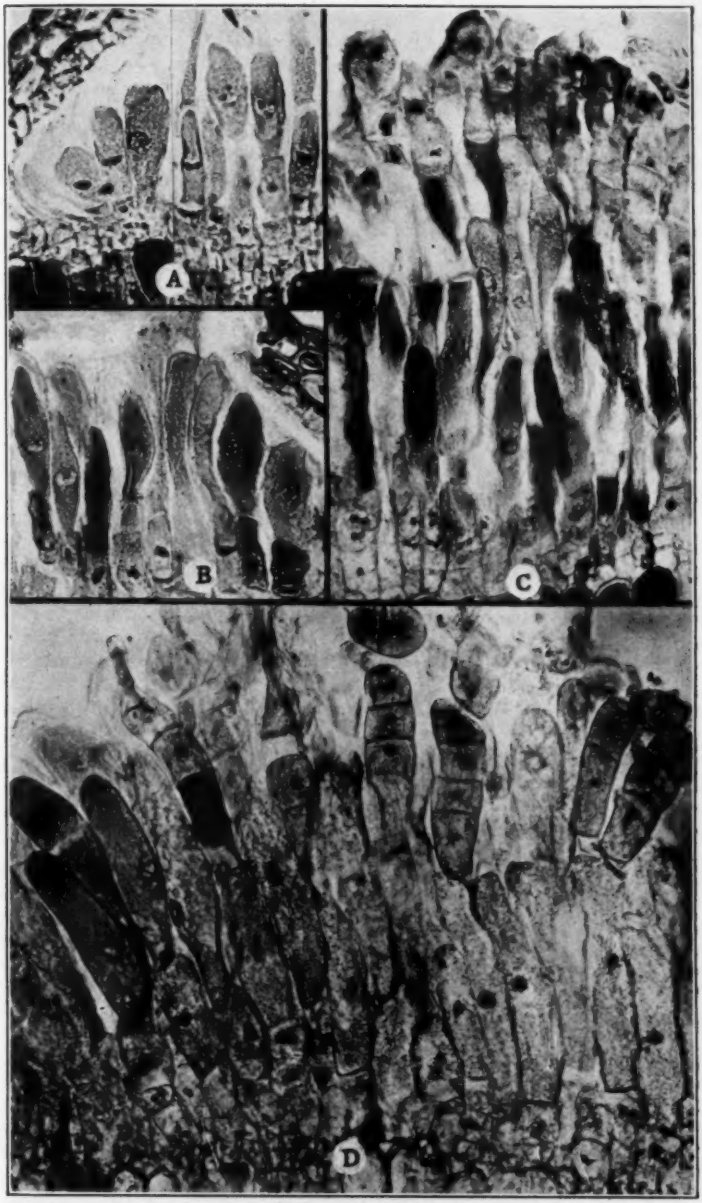
Up to this time there is nothing in the appearance of the cells of the primordium to indicate exactly where cell fusions are to occur. Soon after the epidermis is ruptured, however, the sorus takes on quite a different appearance. Cell fusions which are readily distinguished are seen along a line beneath the point of rupture (fig. 1, B to D). The fusing cells are the second or third cells from the outer ends of the chains, and are therefore intercalary. The writer has not seen fusions between subterminal cells, but this may sometimes occur. Neither are the fusions all on the same level (fig. 1, E). There are more frequently two sterile cells in the chains above the fusing cells (fig. 1, B and D). The conjugations are more readily seen in sections that show the gaps between the lobes of the mesophyll cells. The adjacent walls of the fusing cells quickly disappear, and the two nuclei come closer together (fig. 1, C). The cell thus formed enlarges but its cytoplasm becomes only slightly less vacuolate. At the same time the buffer cells immediately above collapse. The terminal cells of the chains, however, retain their form and persist indefinitely as a sort of peridium, or until cast off with fragments of the ruptured leaf tissue above (pl. 1, C).

CATENULATE SPORES

The fusion cell quickly divides, and the upper daughter cell develops a very dense cytoplasm so that it takes the stains readily. The fusion cell still remains vacuolate. As the sorus ages and increases in size, the buffer cells at the margin elongate correspondingly. The digestive effect which the newly formed spore cells have on this tissue is strikingly demonstrated by the way the fertile layer broadens laterally (pl. 2, A, and fig. 1, E). Although the subterminal cells in the chains are completely destroyed, the terminal cells, at least the outer ends of them, persist, as noted above (pl. 1, C). From this time on, the number of binucleated spore cells cut off in the chain above each fusion cell increases. At the margin, nuclear fusions may occur in the second or third spore of the chain (fig. 1, E); while at the center, there may exist three or four binucleated potential spore cells, above which there are one or two spores, considerably elongated and rich in cytoplasm, but still binucleated (fig. 1, K). Above these the spores are much longer and uninucleated (fig 1, J



A.—Spermogonium of Galloway a
B.—Telial sorus before cell fusions
C.—Sorus showing mature spores, and remains of peridial buffer tissue
(Same magnification in the three figures)



A.—Chains of spores and "peridium" at margin of a sorus
B.—Chains of spores in a young sorus
C.—Spores with stalk-like lower ends, in an old sorus
D.—Spore chains terminated by mature basidia
(Same magnification as in Plate 1)

to L). It would seem that there can be no question that the spores of Gallowaya are catenulate, since it is possible, in a sorus sufficiently developed, to trace the chain upward from the "two-legged" fusion cell below to the germinated spore above (fig. 1, I and L). Ordinarily, then, one could say that at the middle of the mature sorus the chain of cells may be composed of a fusion basal cell; two to four short vacuolated binucleated cells; one or two elongated binucleated spores rich in cytoplasm; one or two long uninucleated spores; and the terminal germinated spore in the form of a four-celled internal basidium (fig. 1, L).

INTERCALARY ELEMENTS

A feature which is brought out more or less diagrammatically in Figure 1, L, *b*, is well shown in Plate 2, D. In some of the chains of cells there is nothing to suggest that these intercalary structures are cells. At H, however, in Figure 1, *d* is a cell which is clearly degenerating, so that one might think that both *b* and *d* are real intercalary cells. C. R. Orton has suggested to the writer that if such were the regular order of development throughout, it might be concluded that the sorus in Gallowaya is a modified aecidium. Such a form would round out an evolutionary series nicely. Until further evidence is found, however, the writer is compelled to consider this intercalary structure, *b*, as formed merely by the elongation of the lower end of the lower cell of the basidium. The wall seems to become separated from the cytoplasmic contents above and elongates in the form of a stalk, pushing the basidium still farther outward. In an aecidium, for example, when the spore is formed, its sister cell is usually sacrificed to serve, as some hold, merely as a disjunctor, or, as others might claim, to make available sufficient food for the maturing of one of the pair. Where the upper daughter cell degenerates and the lower one becomes a spore, the first of these views could not be held. In some cases the intercalary cell elongates to perform the function of a stalk. In Gallowaya, some of the true spores fail to develop, and they may then elongate greatly and function as stalk elements. This intercalary portion seems to be simply the swollen end of the cell wall.

Just how many of the cells in the chain function as spores is problematical. Typically, at any given time only the terminal ones do germinate. After the discharge of the sporidia the walls of the basidium collapse and disappear. Following this there is a sudden stretching or elongation at the lower end of the next spore. There appears to be a limit to the amount of elongation this spore can undergo before processes of disorganization begin. The next spore below, by elongating also, helps to bring the new terminal spore into a favorable position as regards its competitors (fig. 1, I). There is no question that as the sorus ages the lower spores in the chains never succeed in functioning as spores. They are, however, called upon to serve a very useful purpose, for it is only by their sacrifice in excessive elongation that the spore above can be brought into a position favorable for the discharge of its sporidia (fig. 1. I to K). This sacrifice would not be necessary if young spores were being continuously cut off from the basal cell below. In an old sorus, therefore, one frequently finds germinated spores connected directly with the binu-

cleated cells at the base of the sorus by means of a granular aggregation of decomposition products (fig. 1, I) stretched out so that it simulates the expanded stalk of a spore of Gymnosporangium. At different levels on all sides, uninucleated spores are still present (pl. 2, C). Such a picture is misleading, for it suggests that all of the spores are at first sessile, but that by a stretching of the basal part, and as a result of lateral pressures, the spores are brought to different levels.

The spores developed at the margin of the sorus are greatly deformed and truncated (pl. 1, C). Though the terminal ones do germinate to form their internal basidia (fig. 1, H) they can be brought out into the open only by the great elongation of the spore below. Cross walls are not always laid down at right angles to the spore axis. This gives the spore cells the appearance of budding out at the side (fig. 1, G), especially if the wall of the spore above separates from the spore contents and undergoes mucilaginous disorganization.

Each cell of the internal promycelium has a single nucleus. One occasionally finds what might be taken to be two nuclei in each cell (fig. 1, M, N). One of the bodies, which is clearly a nucleus, is larger than the other. The smaller body seems to be more uniform in its composition, and stains less like a nucleus. Good fixation of these stages was not obtained. Binucleated sporidia are often found in the rusts, and it is possible that the original nucleus of the basidial cell sometimes divides precociously instead of waiting until it has passed up into the sporidium.

DISCUSSION

The amount of sterile tissue which is found overlying the sporogenous cells in a rust sorus may not now depend altogether on the depth at which the primordium develops, but it is generally conceded that the location of a sorus with respect to the host tissues did, in evolution, have a considerable influence in determining the type of primordium evolved. Arthur (*2*) and others early recognized the importance of this feature. In some forms of deep-seated aecidia, the primordium is composed of a mass of intertwined hyphae without orientation. The host tissues are crowded aside and, by subsequent disorganization of the fungus tissue at the center, a cavity is developed and food made available for the growth of spores. Where a resistant host tissue above is to be ruptured, one may find that the sorus primordium is composed of a palisade arrangement of hyphal cells thrust against the tissue to be broken away.

Had Lindfors (*13*) studied the organization of the telial sorus in Gymnosporangium and Cronartium and the uredinium in Pucciniastrum and Cronartium, or the deep-seated aecidia in certain Peridermiums, he would no doubt have anticipated the objections that have been raised against the theory that the sterile cells above the fusing cells are the morphological representatives of ancestral red-alga trichogynes, a theory which the propounder himself would no longer support. The nature of the sterile tissue lying above the fertile or fusing cells in a sorus is best understood, not by limiting one's studies of rusts to cell fusions, but by also investigating those sori where no fusions occur. Colley's method of study (*4*) of the white-pine blister rust, *Cronartium ribicola*, is to be commended particularly on this account.

As the writer interprets Colley's discussion of the development of the uredo sorus in *Cronartium ribicola*, the "peridium" is the exact homologue of the buffer tissue in Gymnosporangium. Certain cells of the uredo primordium become oriented vertically, and, by elongating, develop a thrust against the epidermis. These cells divide, and the upper daughter cells, in conjunction with their homologous neighbors, constitute the peridium made up of elongated cells. The subterminal cells now divide to give rise to spore initials above and basal cells below. The telial peridium is evidently developed in much the same way.

It has been found (*5*) that in several species of Gymnosporangium the teleutospores arise from the subterminal cells of the chains composing the primordium. The terminal cells, by swelling and elongating, serve as a buffer tissue to break open the overlying host tissues. The sacrifice of these terminal cells is made necessary by the fact that the tissues above the sorus are very tough, even in the case of leaf sori. The substance originally contained in the cells of the buffer tissue is not wasted, however. The teleutospore buds, by growing up through or between the sterile cells, absorb their predigested disorganization products, just as in all sorts of fungi one sees cases of self-parasitism in the form of "Durchwachsung" phenomena.

In the uredo sorus of Pucciniastrum (*12, 6*) the terminal cells of the sorus primordium are sterile and, though persisting as a "peridium," first lose their contents, swell and elongate, and function primarily as a buffer tissue. Adams (*1*), in a paper on the Peridermiums, shows that the fusing cells are merely intercalary cells in a space making buffer complex of parallel hyphae. In deep-seated sori there may be a half dozen or more cells above the fusing cells in the chain. The writer has found no evidence anywhere in the rusts that these chains are multicellular trichogynes.

Even though a buffer tissue persists more or less, as it does in Gallowaya, it should not be confused with a peridium. Morphologically, the latter structure in the rusts, if one is to take the peridium of the aecidium as the standard, is composed of spore and intercalary cells. In the uredo sorus of Pucciniastrum (*6*) the buffer tissue may be a true peridium, unless Kursanov (*12*) was correct in his statement as to this structure in *Pucciniastrum pirolae*. He claims that the intercalary cells which lie immediately below the terminal cells are cut off by the cells below. This would make the "peridium" of Pucciniastrum the homologue of the "peridium" of the uredinium of Cronartium and of the telium of Gymnosporangium, in neither of which is the structure a true peridium.

There has recently been described (*8*) a short-cycled strain of *Caeoma nitens* in which no spermogonia are developed. In every such case the aecidiospore arising without cell fusion is uninucleated, and on germination produces a two-celled promycelium with only two sporidia. The fact that Gallawaya develops at most only vestigial spermogonia does not seem to be of any particular effect on future growth processes. The internal promycelium is four-celled and produces at least four sporidia. Lindfors (*13*) says that in *Puccinia arenariae*, a short-cycled rust, the teleutospore produces a two-celled promycelium. Cell fusions do not occur in the sorus, the cells of the mycelium being already binucleated. A nuclear fusion in the spores is followed by two divisions, but the septa which divide the

structure into four cells are not laid down. There are two nuclei in each of the cells of the two-celled promycelium, and two nuclei are supposed to enter each sporidium. Lindfors shows two teleutospores germinating with long germ tubes, and he states that Grove had found four-celled promycelia in this species. Evidently this species of rust must receive further attention before it can be compared with the strain of the short-cycled *Caeoma nitens* which develops two-celled promycelia.

In any system of classification based on morphology it may be asked just how important, as showing relationship, is the manner of germination of a spore. Grove (*11*) says, "It is, indeed, doubtful whether the character upon which the Coleosporiacae are united into one group, viz, the internal basidium, is really an indication of close affinity."

If the spores of *Chrysomyxa abietis*, which according to Kursanov (*12*) and Lindfors (*13*) are borne in chains, germinated with internal basidia instead of as they do, would this species be placed in the genus Gallowaya and in the group with Coleosporium? Except for the proliferation of the binucleated cell formed after cell fusion in this Chrysomyxa, there is not very much difference between it and Gallowaya so far as the organization of the sorus is concerned. In both cases the fusing cells are topped by two or three sterile cells. A short chain of binucleated cells is developed after cell fusion. These cells clearly never function as spores; they remain binucleated to the end, representing a short sporophytic generation. Nuclear fusion occurs in the outermost cells, so that a chain of uninucleated teleutospores is formed. Lindfors (*13*) doubts very much whether all of the uninucleated spores function in this Chrysomyxa, since in some cases the lower ones seem to lose most of their cytoplasm and become elongated. This is what often occurs in Gallowaya. In discussions of morphology it is not a question whether a structure functions, or whether a spore germinates, or what form the germ tube takes, but it should be asked, What are its phylogenetic antecedents?

Regardless of the merits of Grove's contention that Ochropsora is not one of the Coleosporiaceae, there can be no question that a system of classification based on the manner of spore germination is not a natural one. The writer (*7*) has shown that in certain strains of *Caeoma nitens* some of the aecidiospores produce long germ tubes which reinfect the host locally so that eventually teleutospores appear in the life cycle, while other aecidiospores, even from the same sorus, produce promycelia directly. So long as an unstable condition exists, such strains of orange-rust of Rubus can not be separated generically.

Galloway (*10, p. 443*) says: "The entire contents of the cell seem to be used up in the formation of the promycelium (sterigma) and the sporidium, and if this is not the case, the formation of secondary sporidia goes on until there is no protoplasm left." W. W. Diehl has informed the writer that he has seen cases where more than one sporidium was formed on the cell outgrowth in Gallowaya. Possibly in rare cases a true external promycelium is formed in Gallowaya. The writer has not seen young spores of this form with more than two nuclei. After germination, however, one can frequently find basidial cells with two bodies, as noted previously, both

very similar to nuclei and lying close together. If two sporidia are to be formed in succession from a single cell, one nucleus must remain behind after the first sporidium has been supplied. So far, this process has not been demonstrated for the rusts, although it is well known in the smuts. Even though two or more sporidia should be formed on the outgrowth from the same cell in Gallowaya, that outgrowth can not be called a promycelium, nor the cell from which it arises a teleutospore. Cells of teleutospores are mother cells, with which reduction divisions are normally associated. In this genus there is a series of binucleated "sporophytic" cells cut off from a fusion cell. Beginning with the oldest cell, the two nuclei fuse in regular order. The fusion nucleus in the oldest spore divides twice, a four-celled structure replacing the uninucleated cell. This may be sufficient evidence for assuming that the binucleated cells below are young spores and the uninucleated cells above them are certainly mature spores. Each four-celled structure is a protobasidium or internal promycelium.

In characterizing Coleosporium, Arthur (*3*), on the basis of what was then known of the telial sorus, says: "Teliospores sessile (by successive formation and displacement due to lateral pressure often appearing catenulate and pedicellate) * * *. By "successive formation" is evidently meant either that a new basal cell displaces the one from which a spore has been cut off, or the original basal cell buds out from the side to form a new spore. It is difficult to understand how the former process could take place once the primordium was organized. As far as the writer is aware, no one has yet reported a Coleosporium in which the basal cell buds out to form a number of spores. It is certain that such methods of spore formation do not occur in Gallowaya. The pedicellate appearance of its spores is in part due to displacement and lateral pressure, but its spores are nevertheless borne in chains. If the more or less persistent sterile tissue enveloping the uredo sori in Hyalopsora, Pucciniastrum, and Cronartium can be called a peridium, then the similar structure in Gallowaya must be referred to by the same designation.

SUMMARY

The mycelial hyphae of *Gallowaya pinicola* are composed of uninucleated cells. Aborted or vestigial spermogonia are sometimes developed between the mesophyll and the overlying hypodermal tissue. Spermatia are seldom formed.

The telial primordium first develops a buffer tissue composed of chains of cells, the terminal ones swelling or elongating somewhat, thus breaking open the overlying host tissues.

Cell fusions occur between intercalary cells in the chains composing the primordium. The fusing cells are the third or fourth cells from the ends.

Several binucleated cells are cut off above the fusion basal cell. These are at least potentially spores. Nuclear fusions occur in regular order, beginning with the oldest binucleated cell. As the terminal spore germinates, the lower end of its cell wall swells and elongates, thus thrusting the protobasidium still farther out in the sorus. Not all of the cells in a chain necessarily function as spores. Some of them become disorganized, owing to excessive elongation.

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SULPHATE CONTENT OF THE LEAF-TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTON[1]

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INTRODUCTION

Previous investigations by the Office of Alkali and Drought-Resistant Plant Investigations and the Office of Biophysical Investigations of the Bureau of Plant Industry, United States Department of Agriculture, have shown that the Egyptian and Upland types of cotton are differentiated not merely by well-marked external botanical characters (*6*)[2] but by the physicochemical properties of their leaf-tissue fluids.

Since these physicochemical properties may be of significance as factors underlying differences in the drought and salt tolerance of the two forms as grown under the saline conditions of the Southwest, it has seemed desirable to investigate them in detail. In a first study (*4*) based on cultures made in 1920 and 1921 it was shown that Pima Egyptian and Meade and Acala Upland cottons are differentiated with respect to the osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration of their leaf-tissue fluids. These results have been confirmed by unpublished studies on these and on other varieties of Egyptian and Upland cotton grown in 1922, 1923, and 1924, and the investigations have been extended to the tissue fluids of the F_1 and F_2 hybrids. The results of these investigations are only partially published.

In their first discussion of the differentiation of the two types of cotton with respect to osmotic concentration as measured in terms of freezing-point depression, Δ, and specific electrical conductivity, κ, the writers pointed out that the higher value of the specific electrical conductivity and the possibly higher value of the ratio of specific electrical conductivity to freezing-point depression, κ/Δ, in the Egyptian type evidence a greater capacity of this type for the absorption and retention in solution of salts derived from the soil.

It has since been shown (*5*) that the chloride content of the leaf-tissue fluids of Pima Egyptian cotton is significantly higher than that of Meade, Acala, and Lone Star Upland cotton grown in immediate association. The present investigation has to do with the sulphate content of the Egyptian and Upland types of cotton.

[1] Received for publication Dec. 24, 1924; issued December, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 661.

MATERIALS AND METHODS

The experimental plants are in part the same as those considered in the earlier investigations. All were grown at the Cooperative Testing Station of the Bureau of Plant Industry in the Gila River Valley at Sacaton, Ariz. The study included:

A. A comparison of Pima Egyptian and Meade Upland cotton grown in 1922. Determinations were based on samples of tissue collected from individual plants.

B. A series of Pima Egyptian and Lone Star Upland plants grown in 1923. Determinations were based on samples taken from groups of plants. This is the series on which the chloride determinations for Pima and Lone Star cotton have been based (*5*).

Collection of samples and methods of preparation of tissue fluids are described elsewhere (*4*, *5*). The sulphate content was determined (*1*) as follows:

Five to ten cubic centimeters of the expressed tissue fluid are placed in an 11.5 cm. porcelain evaporating dish, and 10 cubic centimeters of Benedict-Denis oxidizing reagent [3] are added. This mixture is evaporated to approximate dryness on a water bath and then carefully ignited, at first over a small flame and finally to dull redness for a few minutes. After cooling, the ignition residue is dissolved in dilute hydrochloric acid, the solution filtered, and the sulphate precipitated from the filtrate by the addition of barium chloride and weighed as barium sulphate.[4]

Analyses were based on carefully pipetted samples of 5 to 10 cubic centimeters, preserved in sealed tubes after the addition of a drop of formaldehyde. Concentrations are expressed in terms of grams of sulphate (SO_4) per liter of tissue fluid.

RESULTS

The distribution of sulphate content in class units of 0.5 gm. of sulphate per liter in the single series of determinations made in the cultures of 1922, and in the two series of determinations made on the cultures of 1923, appears in Table I.[5]

[3] This reagent is made by dissolving 25 gms. of crystalline copper nitrate, 25 gms. of sodium chloride, and 10 gms. of ammonium nitrate in enough water to make 100 cubic centimeters of solution. Ten cubic centimeters of the reagent are ample for 10 cubic centimeters of tissue fluid.

[4] It is necessary to make "blank" determination of the reagent and to subtract this from the total weight of barium sulphate. By using high-grade chemicals, a blank of 0.0010 to 0.0035 gm. of barium sulphate is obtained.

[5] In Table II of the paper presenting the results for chloride content in the 1923 cultures, the second series of determinations was divided into Part I and Part II. The sulphate analyses were made for samples taken from the south half of the plot only. Thus the sulphate determinations correspond to Part I only of the chloride series.

TABLE I.—*Frequency distribution of sulphate content (in terms of grams of SO_4 per liter of fluid) in leaf-tissue fluids of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., in 1922 and 1923*

Grams of SO_4 per liter	Cultures in 1922		Cultures in 1923			
	July 25 to Aug. 9		First series, July 29 to Aug. 14		Second series, Aug. 18 to Aug. 31	
	Pima	Meade	Pima	Lone Star	Pima	Lone Star
6.76 to 7.25=7	6					
7.26 to 7.75=7.50	2					
7.76 to 8.25=8	3					
8.26 to 8.75=8.50	2					
8.76 to 9.25=9		1				
9.26 to 9.75=9.50	3		2			
9.76 to 10.25=10	1	1				
10.26 to 10.75=10.50	2	4	2			
10.76 to 11.25=11	2	3	2			
11.26 to 11.75=11.50		1	8		2	
11.76 to 12.25=12		1	15		4	
12.26 to 12.75=12.50		1	12		3	
12.76 to 13.25=13		1	12	1	10	1
13.26 to 13.75=13.50	1	1	7		11	
13.76 to 14.25=14			1	2	15	2
14.26 to 14.75=14.50	2	4	2	5	5	1
14.76 to 15.25=15		1	2	13	7	1
15.26 to 15.75=15.50		3		6	6	7
15.76 to 16.25=16	2		1	9	1	12
16.26 to 16.75=16.50	1		1	4	2	13
16.76 to 17.25=17	1	1		14		7
17.26 to 17.75=17.50				4	1	1
17.76 to 18.25=18				4	1	8
18.26 to 18.75=18.50	1			3	1	4
18.76 to 19.25=19						2
19.26 to 19.75=19.50				1		4
19.76 to 20.25=20		1		1		1
20.26 to 20.75=20.50						
20.76 to 21.25=21		1			1	
21.26 to 21.75=21.50						1
21.76 to 22.25=22						1
22.26 to 22.75=22.50		2				
22.76 to 23.25=23		1				1
23.26 to 23.75=23.50		1				1
23.76 to 24.25=24						1
24.26 to 24.75=24.50						1
	29	29	67	67	70	70

These determinations show that while the sulphate contents of the two types of cotton are highly variable they are quite different, that of the Upland type (Meade and Lone Star) being apparently significantly higher than that of the Egyptian type (Pima).

The variability of the sulphate content is presumably due to the influence of substratum heterogeneity which has been shown to be a practically universal characteristic of experimental fields (*2*, *3*). Illustrations of its influence on the tissue fluids of cotton are sufficiently developed in preceding papers (*4*, *5*). Since the determinations on the two types were made in pairs, the true relationship between the concentrations may be shown best by a double-entry arrangement in tables in which the sulphate content of the two forms are shown on two axes. The form of table is identical with that employed in the writers' discussion of chloride content (*5*).

Table II presents the results for the first comparison of Pima and Meade plants (1922). Tables III and IV give the results for the first and second series of determinations on the Pima Egyptian and Lone Star Upland cultures of 1923.

TABLE II.—*Comparison of sulphate content of Pima Egyptian and Meade Upland cotton grown under irrigation at Sacaton, Ariz., 1922*

MEADE	7.00	7.50	8.00	8.50	9.00	9.50	10.00	10.50	11.00	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	17.00	17.50	18.00	18.50	Totals
		2	3	2		3	1	2	2					1		2			2	1	1			1	29
23.50														1											1
23.00																				1					1
22.50																1					1				2
22.00																									
21.50																									
21.00																								1	1
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18.00																									
17.50																									
17.00				1																					1
16.50																									
16.00																									
11.50		1	1					1																	3
15.00							1																		1
14.50	2								2																4
14.00																									
13.50			1																						1
13.00				1																					1
12.50						1																			1
12.00						1																			1
11.50	1																								1
11.00		1				1		1																	3
10.50	1		1													1			1						4
10.00	1																								1
9.50																									
9.00	1																								1
8.50																									
8.00																									
7.50																									
7.00																									
Totals																									

PIMA

TABLE III.—*Comparison of sulphate content of Pima Egyptian and Lone Star Upland cotton grown under irrigation at Sacaton, Ariz., in 1923. First series of determinations*

LONE STAR	9.50	10.00	10.50	11.00	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	Total
Total	2		2	2	8	15	12	12	7	1	2	2		1	1	67
20.00																
19.50						1										1
19.00								1								1
18.50																
18.00				1	1	1		2			1				1	3
17.50			1		1		1									4
17.00				1	1	2	3	5	2			1				4
16.50			1				2		1							14
16.00					1	1	2	2	2	1						4
15.50	1					4								1		9
15.00	1				2	2	3	2	2			1				6
14.50						4	1									13
14.00					2											5
13.50																2
13.00											1					
12.50																1
12.00																
11.50																
11.00																
10.50																
10.00																
9.50																

PIMA

TABLE IV.—*Comparison of sulphate content of Pima Egyptian and Lone Star Upland cotton grown under irrigation at Sacaton, Ariz., 1923. Second series of determinations*

LONE STAR	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	17.00	17.50	18.00	18.50	19.00	19.50	20.00	20.50	21.00	Total
Total..	2	4	3	10	11	15	5	7	6	1	2		1	1	1					1	70
24.50										1											1
24.00									1												1
23.50														1							1
23.00								1													1
22.50																					
22.00																					
21.50									1												1
21.00							1														1
20.50																					
20.00						1															1
19.50							1		1		1		1								4
19.00				1					1												2
18.50				2					1											1	4
18.00		1	1	1	1	1		1	1		1										8
17.50						1															1
17.00				2	1	4															7
16.50		1		2	4	2	2	2													13
16.00			1	1	4	3	1	2													12
15.50	2		1	1	1	2															7
15.00								1													1
14.50		1																			1
14.00		1				1															2
13.50																					
13.00															1						1
12.50																					
12.00																					
11.50																					
	11.50	12.00	12.50	13.00	13.50	14.00	14.50	15.00	15.50	16.00	16.50	17.00	17.50	18.00	18.50	19.00	19.50	20.00	20.50	21.00	Total

PIMA

Except for a few outlying analyses,[6] the sulphate content of the Upland type is higher than that of the associated Egyptian plants. This is shown by the fact that practically without exception the sulphate contents of the Upland variety lie above the diagonal cells (marked by the short rules) about which they should be concentrated if the content were the same in the two types.

Determining the coefficients of correlation between the sulphate content of associated Egyptian and Upland plants, the values are set forth in Table V.

TABLE V.—*Correlation between sulphate content of associated plants or groups of plants of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., in 1922 and 1923*

	Table	Correlation coefficient and probable error, $r \pm E_r$	Ratio of correlation to probable error, r/E_r
Comparison of Pima Egyptian and Meade Upland cotton, 1922:			
First series, July 25 to Aug. 9, correlation between Pima and Meade.	II	0.6521±0.0720	9.06
First comparison of Pima Egyptian and Lone Star Upland cotton, 1923:			
First series, July 29 to Aug. 14. Correlation between Pima and Lone Star	III	.1047± .0815	1.28
Second comparison of Pima Egyptian and Lone Star Upland cotton, 1923:			
Second series, Aug. 18 to Aug. 31. Correlation between Pima and Lone Star	IV	.3970± .0679	5.85

The three coefficients are all positive, and two of them may be considered statistically significant in comparison with their probable errors. They are, however, more irregular in actual magnitude than those demonstrated for chloride content (*5*).

The positive correlation indicates that the sulphate contents of associated Egyptian and Upland plants are influenced in a similar manner by some common extrinsic factor. It also indicates the necessity for considering the correlation between associated determinations in the calculation of the probable error of the difference between them.

The mean sulphate contents of the individual series and their probable errors, the differences in the mean sulphate content of the Egyptian and Upland types, and the probable errors of these differences appear in Table VI. The probable errors of the differences are calculated with due regard to the correlations between the variables by the formula

$$\sigma^2_{(p-u)} = \sigma^2_p + \sigma^2_u - 2r_{pu}\,\sigma_p\,\sigma_u$$

The differences are from 9 to 26 times as large as their probable errors, and hence are clearly significant. Absolute differences are, on the average, 3 to 4 grams of sulphate per liter. Percentage differences, calculated by using the sulphate content of the Upland type as a base, range from about 18 to about 28 per cent.

[6] These exceptions are presumably due to experimental errors but are included in order to avoid any possibility of the selection of data in the formulation of the conclusion.

TABLE VI.—*Mean sulphate content (in terms of grams of SO_4 per liter) of tissue fluids of Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz., 1922 and 1923*

	N	Mean sulphate content for Egyptian cotton	Mean sulphate content for Upland cotton	Difference between Egyptian and Upland cotton		
				Absolute difference and probable error	Ratio of absolute difference to probable error	Percentage difference
Comparison of Pima Egyptian and Meade Upland cotton, 1922:						
First series, July 25 to Aug. 9. Comparison of Pima and Meade	29	10.5517±0.4447	14.6724±0.5385	−4.1206±0.4189	9.84	28.08
First comparison of Pima Egyptian and Lone Star Upland cotton, 1923:						
First series, July 29 to Aug. 14. Comparison of Pima and Lone Star	67	12.5373± .1022	16.2164± .0801	−3.6791± .1410	26.10	22.69
Second comparison of Pima Egyptian and Lone Star Upland cotton, 1923:						
Second series Aug. 18 to Aug. 31. Comparison of Pima and Lone Star	70	14.1642± .1294	17.3357± .1793	−3.1714± .1750	18.12	18.29

SUMMARY AND CONCLUSION

The sulphate content of the leaf-tissue fluids of Meade and Lone Star Upland cotton has been investigated in comparison with that of Pima Egyptian cotton as grown under irrigation in the Gila River Valley, Sacaton, Ariz.

The sulphate content of the Upland varieties (Meade and Lone Star) is higher than that of the Egyptian variety (Pima). The differences are clearly significant in comparison with their probable errors and range from 3 to 4 grams per liter, or from 18 to 28 per cent of the Upland value.

These results as compared with those of the earlier study of chloride content show that, as far as the varieties investigated are concerned, the behavior of these two types of cotton with respect to the absorption of chlorides and sulphates is quite different, the Egyptian type taking up larger quantities of chlorides, and the Upland types absorbing larger quantities of sulphates.

The relationship between the concentration of these ions in the plant, the relation of their concentration in the plant to that of their concentration in the soil solution, and their behavior in inheritance are under investigation.

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ABORTION-BACTERIN TREATMENT OF COWS HAVING UDDERS INFECTED BY BACTERIUM ABORTUS[1]

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INTRODUCTION

For a number of years investigators of bovine infectious abortion have recognized that when cows become infected with *Bacterium abortus* the microorganism shows a tendency to localize in the udder and to live and multiply in this organ for widely varying periods of time. It is also common knowledge that *Bact. abortus* readily thrives in the uteri of pregnant bovines, but is seldom found in this organ longer than two months after the expulsion of a fetus, living or dead. Schroeder and Cotton,[2] in their efforts to determine whether the organism becomes localized in the cow's body elsewhere than in the organs mentioned, found the infection present in the supramammary lymph glands and certain other lymph glands at the brim of the pelvis. The present writers were able in a single instance to isolate the infection from the femoro-tibial joint of a cow; lameness in this joint prompted the search for the organism in this particular location.

Although in rare instances the infection has been found localized in organs other than the uterus, udder, and associated lymph glands of the latter, it seems highly probable that it is commonly the udder which harbors the infection in the animal between gestation periods, and acts as a source of infection for the uterus when conditions are favorable for the multiplication of *Bact. abortus* therein. When second and third abortions in the same animal are observed as being associated with *Bact. abortus*, and placental infection with this organism in those cases which harbor the infection is common, even though a living calf is produced, it seems that the devising of a method whereby this focus of infection may be eliminated would accomplish much toward solving the abortion problem. Some investigators have been of the opinion that success in such an undertaking may not be unattainable. Experimental use has been made of certain drugs which, it was thought, might possibly exert antiseptic action during their elimination through the udder. Results from such medication seem to have yielded but slight encouragement so far.

In this paper are discussed the procedure followed and the results obtained in an endeavor to overcome *Bact. abortus* udder infection by repeated subcutaneous administrations of *Bact. abortus* bacterin. In undertaking this line of treatment it did not appear wholly unreasonable to infer that any immunity which might be engendered by such treatment would have a desirable effect at the seat or seats of the infection.

[1] Received for publication Nov. 21, 1924; issued December, 1925.

[2] Schroeder, E. C., and Cotton, W. E. Some facts about abortion disease. Jour. Amer Vet. Med. Assoc. (n. s. 3) 50: 321-330. 1916.

EXPERIMENTAL PROCEDURE

The experimental work was carried out in a herd of approximately 200 head of dairy cattle in which infectious abortion had prevailed for about five years. An agglutination test of the herd, made March 17, 1922, showed that the blood serum of 22 cows caused clumping of a *Bact. abortus* suspension in amounts as small as 0.001 c. c., and that the end point in the case of 8 others was 0.002 c. c. Results of this test on these 30 cows are given in Table I.

TABLE I.—*Blood-serum reactions obtained in Bacterium abortus agglutination tests on 30 cows*

Cow No.	Quantity of blood serum (cubic centimeters)						Cow No.	Quantity of blood serum (cubic centimeters)					
	0.04	0.02	0.01	0.005	0.002	0.001		0.04	0.02	0.01	0.005	0.002	0.001
17	+	+	+	P	P	S	432	+	+	+	+	+	+
49	+	+	+	+	+	+	434	+	+	+	+	+	+
51	+	+	+	+	S	S	435	+	+	+	+	+	P
54	+	+	+	+	S	S	439	+	+	+	+	+	+
67	+	+	+	+	+	+	443	+	+	+	+	+	+
71	+	+	+	+	+	P	445	+	+	+	+	+	+
84	+	+	+	+	P	S	465	P	P	P	P	P	P
89	+	+	+	+	+	P	50	+	+	+	+	+	−
223	+	+	+	+	+	+	247	+	+	+	+	+	−
245	+	+	+	+	+	+	421	+	P	S	S	S	−
254	+	+	+	+	+	+	441	+	+	+	+	+	−
409	+	+	+	+	+	+	443	+	+	P	P	S	−
417	+	P	P	P	S	S	448	+	P	S	S	S	−
418	+	+	+	+	+	+	466	P	P	P	P	P	−
431	+	+	+	+	+	+	468	S	S	S	S	S	−

+ = Pronounced agglutination. S = Slight agglutination.
P = Partial agglutination. − = No agglutination.

In making these agglutination tests six different quantities of blood or milk serum were used. There is placed in six different test tubes these respective amounts: 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 c. c. There is then added to each tube 1 c. c. of a 0.5 per cent carbolized suspension of *Bact. abortus* similar in density to a barium-sulphate suspension resulting from the addition of 1 c. c. of a 1: 100 solution of barium chloride to 99 c. c. of a 1:100 solution of sulphuric acid. Results are indicated by these signs: +, which signifies pronounced agglutination of the bacteria; P, clumping incomplete; S, a trace of agglutination; and −, no agglutination. Readings of the tests were made after incubation for 36 hours.

After identifying the marked reactors to the abortion test, composite samples each containing 40 to 50 c. c. of milk or udder secretions, were taken from these 30 animals, as were blood specimens also, with the idea of detecting those cows which carried the infection in their udders.

The milk was kept in the refrigerator over night, after which the cream layer was pipetted off. The specimens were then thoroughly shaken, warmed to body temperature, and injected intra-abdominally into guinea pigs in 5 c. c. amounts. Two guinea pigs were used in most cases for making this preliminary test; in two or three cases four were inoculated. In obtaining the agglutinin titer of the milk, a small quantity of rennet was used to curdle the specimens. Milk

serum was used because it was more satisfactory to read the agglutination tests from it than from the milk.

It was suspected that success or failure in infecting the guinea pigs might depend to some extent upon the quantity of milk the cows gave at the time the milk specimens were taken, or, in other words, upon the concentration of the bacteria; so a record was kept of the quantity of milk produced by each animal during the 24-hour period previous to the collection of the various samples.

A practice was made of killing the inoculated guinea pigs in from five to eight weeks after the intra-abdominal injections. Serum-agar slants in nearly every case were sown with spleen tissue as a means of detecting *Bact. abortus* infection, and the cultures were subjected to incubation at 37° C. in closed jars in which 10 per cent of atmosphere was displaced by carbon-dioxide gas.

Table II shows the quantity of milk produced by each of the 30 cows during the 24-hour period just prior to the collection of the samples, the agglutination results with blood and milk sera, and outlines the procedure followed in inoculating, autopsying, and culturing the guinea pigs.

The results obtained from the milk inoculations of guinea pigs proved that 17 of the 30 cows carried *Bact. abortus* in their udders. It is probable that the number of carriers was considerably larger than was indicated by the inoculation results. The number of guinea pigs used for each case was small, and death from enteritis, pneumonia, etc., was of frequent occurrence—factors which may have rendered the results somewhat misleading.

Sixteen of the udder-infected cows were used for testing the value of the abortion-bacterin treatment. This number was divided equally into two groups. In order to make the division as nearly uniform as possible, an effort was made to include in each group animals which had been carriers for similar lengths of time as based on their histories and records of previous serological tests.

The eight cows of one group (Nos. 51, 247, 409, 432, 435, 439, 442, and 468) were each given a subcutaneous injection of 10 c. c. of abortion bacterin on the following dates: May 24, May 29, June 3, June 9, June 15, and June 21 (1922). The bacterin was prepared from numerous strains of *Bact. abortus* which had been under artificial cultivation for a year or longer. The organisms were killed by placing the flasks containing the suspensions in a water bath maintained at a temperature of 60° C. for one-half hour. The bacterin consisted of approximately one billion organisms per cubic centimeter. The eight animals of the second group were used as controls.

In order to determine whether there was any systematic disturbance resulting from such bacterin injections which might prove detrimental to the animals from the standpoint of production or otherwise, temperatures of the treated cows were taken on the days that the injections were made and three times daily on the four following days. Their daily milk yield was also recorded. There was usually a slight reduction in milk-yield on the day following the treatment and sometimes on the second day. A slight rise of temperature was observed in a few of the cows following the injections. In some of the animals there was as welling at the point of inoculation that did not entirely disappear for a week or ten days.

TABLE II.—*Data of agglutination and inoculation tests of blood and milk samples from the 30 cows tabulated in Table I*

Cow No.	Milk yield in pounds[a]	Agglutination results[b]		Guinea-pig inoculations[c]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
17	9.8	+ + + P P P	P P P P S −	74799	.....	May 7	+ P P P P −	Spleen much enlarged	Positive.
				74900	.....	do	+ P P S S −	No visble lesions	Do.
49	Dry.	+ + + + P P	+ + + + + +	74777	Apr. 8	.....	(d)	do	Not cultured.
				74370	Apr. 18	.....	− − − − − −	do	Negative.
51	47	+ + + P S S	+ P P S − −	74901	.....	May 7	+ + P P P −	Spleen enlarged	Positive.
				74902	.....	do	+ + + + P P	No visible lesions	Do.
54	26.5	+ + P P S S	+ P P S − −	74795	.....	do	− − − − − −	do	Negative.
				74796	.....	do	− − − − − −	do	Do.
67	Dry.	+ + + + + +	.....	74787	Apr. 12	.....	(d)	No Bact. abortus lesions	Not cultured.
				74788	.....	May 7	+ + + + P P	Spleen enlarged	Positive.
71	42.7	+ + + + P P	+ + P S − −	74905	.....	do	P P P P P P	No Bact. abortus lesions	Do.
				74906	Apr. 11	.....	(d)	do	Negative.
84	Dry.	+ + + + + S	P P P P P P	74341	.....	May 7	+ + P P − −	No visible lesions	Positive.
				74342	.....	do	− − − − − −	do	Negative.
89	7.5	+ + + + + P	+ + + P P S	74783	Apr. 20	.....	(d)	No Bact. abortus lesions	Do.
				74784	Apr. 19	.....	(d)	do	Do.
223	Dry.	+ + + + + +	+ + + P P S	73105	.....	May 7	+ + P P P −	Spleen much enlarged	Positive.
				73107	Apr. 1	.....	(d)	No Bact. abortus lesions	Not cultured.
				74912	.....	May 7	P P P P S −	Spleen enlarged and nodular	Positive.
				74913	.....	do	+ − − − − −	No visible lesions	Negative.
245	44.5	+ + + + + +	+ + P S − −	74772	Apr. 2	.....	(d)	No Bact. abortus lesions	Not cultured.
				74773	.....	May 7	− − − − − −	No visible lesions	Negative.
254	30	+ + + + + +	+ + + + S −	74791	.....	do	+ + + P P −	Spleen enlarged	Positive.
				74792	Apr. 10	.....	(d)	No Bact. abortus lesions	Negative.
409	34.4	+ + + + + +	+ P P P S −	74764	.....	May 7	P P S S − −	Spleen slightly enlarged	Positive.
				74765	Apr. 26	.....	(d)	No Bact. abortus lesions	Not cultured.
				74602	.....	May 1	− − − − − −	do	Negative.
				74603	.....	do	− − − − − −	do	Do.
417	17.6	+ P P P S S	− − − − − −	74785	.....	May 7	− − − − − −	No visible lesions	Do.
				74786	Apr. 13	.....	(d)	No Bact. abortus lesions	Do.
418	33.2	+ + + + P P	+ + + P − −	74781	Apr. 7	.....	(d)	do	Do.
				74782	Apr. 1	.....	(d)	do	Not cultured.
				74910	Apr. 20	.....	(d)	do	Negative.
				74911	May 7	.....	− − − − − −	do	Do.
431	17.6	+ + + + + +	P S − − − −	74739	.....	May 7	− − − − − −	No visible lesions	Do.
				74740	.....	do	− − − − − −	do	Do.
432	24.4	+ + + + + +	+ + + + P P	74770	.....	do	− − − − − −	do	Do.
				74771	.....	do	+ P P − − −	do	Positive.

434	20.1	+ + + + + +	+ + + + P P	74789	Apr. 19		(d)	No Bact. abortus lesions	Not cultured.
				74790	May 7			do	Negative.
435	34.6	+ + + + P S	+ + + P S −	74766		do		No visible lesions	Positive.
				74767		do		do	Negative.
				74337		do	P P P P − −	Spleen much enlarged	Positive.
				74338		do		No visible lesions	Negative.
439	17.7	+ + + + + +	+ + + P S −	74797		do		do	Do.
				74798		do	P P S	do	Positive.
442	34	+ + + + + +	+ + + P S −	74768		do		do	Do.
				74769		do		Spleen slightly enlarged	Do.
445	23.4	+ + + + + +	+ + P P S −	74601		do		No visible lesions	Negative.
				74336		do		do	Do.
465	21.8	P P P P P P	+ + + + S −	74908		do	S	Spleen slightly enlarged	Positive.
				74909		do	+ + P P	No visible lesions	Do.
				74914		do	+ P P S	Spleen enlarged	Do.
50	18.3	+ + + + + −	+ + + P − −	74903		do		No visible lesions	Negative.
				74904		do		do	Do.
247	49.3	+ + + + + −	+ + P S − −	74762		do	+ + + + P P	do	Positive.
				74763		do	+ + + + P S	Spleen slightly enlarged	Do.
421	7.9	+ P S S S −	+ P P S − −	74907		do		No visible lesions	Negative.
				74776		do		do	Do.
441	28.6	+ + + + + −	+ + + P S −	74777		do	+ + + + P −	Spleen slightly enlarged	Positive.
				74778		do	+ + P P − −	do	Do.
443	25.7	+ + P P S −	S S S − − −	74760		do		No visible lesions	Negative.
				74761	Apr. 20		(d)	No Bact. abortus lesions	Do.
448	9.8	+ P S S S −	+ + P S − −	74779	May 7			No visible lesions	Do.
				74780		do		do	Do.
466	24.7	P P P P P −	P P P S − −	74775		do	+ P P P S −	Spleen slightly enlarged	Positive.
				74776	Apr. 13		(d)	do	Negative.
468	23.1	S S S S S −	P S S S − −	74793	Apr. 25		(d)	Abscess epididymis	Positive
				74794	May 7		+ P P P − −	Spleen enlarged	Do.

[a] For the 24 hours (Mar. 28, 1922) just prior to taking samples.

[b] In this and following tables the quantities of blood or milk sera used in the agglutination tests were 0.04, 0.02, 0.01, 0.005, 0.002, and 0.001 c. c., respectively. For explanation of symbols, see footnote to Table I.

[c] All inoculated March 29, 5 c. c. milk per pig.

[d] No test.

[e] "No *Bact. abortus* lesions" signifies no perceptible enlargement or nodular condition of spleen, absence of visible necrotic foci in liver, absence of visible lesions involving testicles, epididymi, bones, articulations, etc.

Table III shows the temperature reactions and variations in milk yield that followed the initial treatment. They are more or less typical of the temperature and milk yield variations that occurred following the subsequent bacterin injections.

TABLE III.—*Temperatures and milk yield of cows for four days following first abortion-bacterin treatment on May 24, 1922*

Cow No.	Date	8 a. m.	12 m.	4.30 p. m.	Milk yield	Cow No.	Date	8 a. m.	12 m.	4.30 p. m.	Milk yield
		° *F.*	° *F.*	° *F.*	*Pounds*			° *F.*	° *F.*	° *F.*	*Pounds*
51	May 24	------	101.0	101.1	46.7	435	May 24	------	------	101.0	29.1
	May 25	103.7	103.2	103.0	43.3		May 25	101.8	101.6	101.4	25.9
	May 26	103.2	101.6	101.8	45.3		May 26	101.6	101.8	101.7	29.8
	May 27	101.8	101.7	102.0	44.6		May 27	101.5	101.4	101.8	29.5
	May 28	101.9	102.0	102.0	47.3		May 28	101.2	101.2	101.0	28.2
247	May 24	------	------	100.6	52.5	439	May 24	------	100.8	100.8	16.8
	May 25	103.4	103.0	101.4	49.7		May 25	101.5	101.8	101.5	17.7
	May 26	101.0	101.4	101.5	50.9		May 26	101.6	101.5	101.6	16.3
	May 27	101.2	101.2	100.6	52.2		May 27	101.7	101.7	101.8	18.6
	May 28	101.0	101.2	101.0	51.0		May 28	101.6	101.7	101.8	17.5
442	May 24	------	------	101.2	23.9	409	May 24	------	------	101.0	32.9
	May 25	101.8	101.6	101.4	22.6		May 25	100.8	100.9	101.0	31.0
	May 26	101.0	101.3	101.2	23.2		May 26	100.6	100.9	101.3	33.1
	May 27	101.2	101.2	101.4	23.2		May 27	101.2	101.2	101.0	31.6
	May 28	101.5	101.4	101.3	22.8		May 28	101.3	101.4	101.0	31.1
432	May 24	------	------	101.2	22.0	468	May 24	------	100.4	100.3	21.4
	May 25	102.0	101.8	101.5	19.3		May 25	100.8	101.3	101.4	22.2
	May 26	101.2	101.2	101.5	19.4		May 26	101.9	101.7	101.8	20.3
	May 27	101.1	101.2	101.4	21.5		May 27	101.8	102.0	102.0	20.9
	May 28	101.5	101.4	101.0	20.8		May 28	101.8	102.0	101.9	22.2

Composite samples of milk were obtained from animals of the treated and control groups on six different dates following the administration of the bacterin, with the object of determining whether the bacterin injections had exerted curative action. Blood samples were also usually taken on the same dates. The blood and milk samples collected on the various dates were handled in a manner similar to that described for the original collection. The periods that intervened between the completion of the bacterin treatments and the collection of the different samples were approximately 2, 4, 6, 11, 15, and 20 months.

Tables IV to XII, inclusive, show the manner in which the blood and milk samples reacted to the agglutination test, results of the milk inoculations of guinea pigs, the serological and cultural results obtained from the inoculated guinea pigs, and other data.

DISCUSSION OF RESULTS

In the writers' studies of the results of numerous guinea-pig inoculations it was found that the nature of the agglutination results of the blood serum, five to eight weeks after inoculation, was in most cases a reliable index to the presence or absence of *Bacterium abortus* infection, and that to depend wholly upon macroscopic lesions as indicators of such infections may in many cases prove misleading. This was clearly evidenced by the fact that it was frequently possible to isolate from 150 to 200 colonies of the abortion organism from spleens of inoculated guinea pigs which were normal in their gross arance. In only a few instances did reacting guinea pigs give ive cultural results.

In the first guinea-pig inoculation test following the bacterin treatment of the cows it was demonstrated that 7 treated and 5 control cows were eliminating the abortion organism in their milk; and in the second series of inoculations, 4 treated and 6 control cows showed infected udders. Before the third test was made one of the treated cows (No. 51) died. The third test indicated that 4 treated and 4 control cows were carriers of the microorganism. Five treated and 5 control cows produced milk that infected guinea pigs on the fourth test; 3 treated cows and 5 controls gave milk from which guinea pigs were infected on the fifth test; and 3 treated cows and 5 controls gave milk from which guinea pigs were infected on the sixth test.

The inoculation results indicated that the guinea-pig method of detecting *Bact. abortus* infection is by no means ideal or perfect. Not only was there a lack of uniformity in the results of the inoculation tests of milk of certain cows collected on different dates—irregularities which may have been due to an intermittent appearance of the infection in the milk—but the same sample of milk when administered to several guinea pigs in the same quantities frequently yielded conflicting results. The guinea-pig cultural results recorded in Table X plainly show that volumetrically equal doses of material containing *Bact. abortus* do not always affect the animals in a similar manner.

The number of bacterin-treated cows which continued to be carriers of *Bact. abortus* 608 days after the immunizing treatment, as indicated by the inoculation results, was 3, or approximately 43 per cent, while the number of controls was 5, or 62½ per cent. Although the number of carriers after the treatment was larger in the control group, the difference did not appear to be sufficiently marked to justify the conclusion that the bacterin injections had any appreciable effect in overcoming the udder infection. Table XI shows the agglutination reactions that were obtained with blood serum from the 16 cows obtained on the different dates, and Table XII shows the agglutination reactions with the milk whey from the same animals, as well as the milk yield for a 24-hour period preceding the collection of the samples. The blood reactions, while showing some variation in intensity on the different dates of collection, are more uniform, possibly, than might be expected when it is considered that a number of the cows, including Nos. 432, 17, 84, and 441, ceased, as far as the guinea-pig tests showed, to carry the infection in their udders for a period of from one to one and a half years.

During the experiment comparatively few abortions occurred, while barrenness troubles were rather pronounced. The 15 cows which were carried through the experiment, continuing approximately two years, have produced 16 living calves. Five cows are now (April 3, 1924) heavy with calf. Cow 439 aborted a fetus in which *Bact. abortus* was present. Cow 84 aborted twice, the presence of *Bact. abortus* being demonstrated in the first abortion, but failure was experienced in isolating the organism in the second. Two of the cows (465 and 468) became pregnant only after ten services. Cow 247 seems to be permanently barren. Several other cows were bred five to six times before conception occurred. Table XIII gives some breeding data relative to the group since the experiment was undertaken.

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TABLE IV.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 63 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds[a]	Agglutination results		Guinea-pig inoculations[b]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
51	18.2	+ + + + S −	+ + P S − −	76089	----------	Nov. 2	− − − − − −	No visible lesions	Negative.
				76090	----------	---do----	− − − − − −	---do----	Do.
				76091	----------	---do----	− − − − − −	---do----	Do.
247	----------	+ + + + + +	+ + P S − −	76046	----------	---do----	− − − − − −	---do----	Do.
				76047	----------	---do----	+ + + + S −	Spleen enlarged	Positive.
				76048	----------	---do----	− − − − − −	Spleen slightly enlarged	Do.
409	25	P P P + + +	+ + + + − −	76040	----------	---do----	P S S S − −	No visible lesions	Do.
				76041	----------	---do----	− − − − − −	---do----	Negative.
				76042	----------	---do----	− − − − − −	---do----	Do.
432	18.6	+ + + + + +	+ + P P − −	76095	----------	---do----	P P P S − −	Spleen slightly enlarged	Positive.
				76096	----------	---do----	+ P P P S −	No visible lesions	Do.
				76097	----------	---do----	− − − − − −	---do----	Negative.
435	27.5	S S S S P P	+ S S − − −	76092	----------	---do----	+ + + P P S	Spleen enlarged; liver lesions	Positive.
				76093	----------	---do----	− − − − − −	No visible lesions	Negative.
				76094	----------	---do----	P − − − − −	Liver lesions; spleen normal	Positive.
439	14	P P + + + +	+ + + + P S	76086	----------	---do----	+ + + + + P	No visible lesions	Do.
				76087	----------	---do----	− − − − − −	---do----	Negative.
				76088	----------	---do----	− − − − − −	---do----	Do.
442	17.4	P P P P + +	+ P P S − −	76043	----------	---do----	− − − − − −	---do----	Do.
				76044	----------	---do----	S S S − − −	---do----	Positive.
				76045	----------	---do----	+ P P S − −	Spleen enlarged; necrotic foci in liver	Do
468	17.2	P P P + + S	+ + P S − −	76080	----------	---do----	P S − − − −	Spleen nodular; liver lesions	Do.
				76081	----------	---do----	− − − − − −	No visible lesions	Negative.
				76082	----------	---do----	P P P − − −	Spleen and liver lesions	Positive.

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17	18.3	P P S S S S	+ P P P S −	76083	.....	Nov. 2	− − − − − −	No visible lesions	Negative.
				76084	.....	do	− − − − − −	do	Do.
				76085	.....	do	− − − − − −	do	Do.
71	18.3	P P + + + +	+ P P P S −	76518	.....	do	+ + + + + +	Spleen nodular; liver lesions	Positive.
				76519	.....	do	+ + + P P P	Liver lesions; spleen normal	Do.
				76520	.....	do	+ + + + P −	Spleen enlarged; liver lesions	Do.
84	24	+ + P P P P	+ + P S − −	76527	.....	do	− − − − − −	No visible lesions	Negative.
				76528	.....	do	− − − − − −	do	Do.
				76529	.....	do	− − − − − −	do	Do.
223	51	P + + + + +	+ + + S − −	76524	.....	do	− − − − − −	do	Do.
				76525	.....	do	+ + + + P P	Spleen and liver lesions	Positive.
				76526	.....	do	P P + P S −	No visible lesions	Negative.
254	19.6	+ + + + P P	+ + + + P −	76530	.....	do	+ + + P P P	Spleen enlarged	Positive.
				76531	.....	do	+ + P S − −	No visible lesions	Do.
				76532	.....	do	P P P P − −	do	Do.
441	19.5	+ + + + P P	P P P − − −	76533	.....	do	− − − − − −	do	Negative.
				76534	.....	do	− − − − − −	do	Do
				76535	.....	do	− − − − − −	do	Do.
465	19,6	P + + + P −	+ + S − − −	76521	Sept. 13		(c)	No autopsy	Not cultured.
				76522	.....	Nov. 2	− − − − − −	Spleen and liver lesions	Positive.
				76523	.....	do	− − − − − −	No visible lesions	Negative.
466	Dry.	+ P P P P P	+ + + + + +	76536	.....	do	+ + + P P P	Spleen and liver lesions	Positive.
				76537	.....	do	P P P S S −	Spleen enlarged	Do.
				76538	.....	do	+ + + + + +	Spleen and liver lesions	Do.

[a] For the 24 hours (Aug. 23, 1922) just prior to taking sample.
[b] All inoculated Aug. 24, 1922, 8 c. c. milk per pig.
[c] No test.

TABLE V.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 110 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds[a]	Agglutination results		Guinea-pig inoculations[b]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
51	21.6	+ + + + + P	P P P P S −	76962	----------	Nov. 24	− − − − − −	No visible lesions	Negative.
				76963	----------	---do----	− − − − − −	---do----	Do.
				76964	----------	---do----	− − − − − −	---do----	Do.
247	18.5	+ + + + + +	+ P P P − −	76977	Oct. 23	----------	(c)	No autopsy	Not cultured.
				76978	----------	Nov. 24	+ + + + + +	Spleen and liver lesions	Positive.
				76979	----------	---do----	+ + P P − −	Spleen slightly enlarged	Do.
432	15.4	P P P + + +	P P P P P P	76953	Nov. 17	----------	(c)	No Bact. abortus lesions	Not cultured.
				76954	----------	Nov. 24	− − − − − −	---do----	Negative.
				76955	----------	---do----	− − − − − −	---do----	Do.
435	26	+ P P P P P	+ P P − − −	76971	Oct. 17	----------	(c)	No autopsy	Not cultured.
				76972	----------	Nov. 24	− − − − − −	No visible lesions	Negative.
				76973	----------	---do----	− − − − − −	---do----	Do.
439	20.1	P P + + + +	+ + P P − −	76959	----------	---do----	− − − − − −	---do----	Do.
				76960	----------	---do----	− − − − − −	---do----	Positive.
				76961	----------	---do----	− − − − − −	---do----	Negative.
409	20.7	+ + + + + +	+ + + − − −	76968	----------	---do----	− − − − − −	---do----	Positive.
				76969	----------	---do----	+ + + P − −	---do----	Do.
				76970	----------	---do----	− − − − − −	---do----	Negative.
442	15.1	P P P P + P	+ + P S S −	76950	----------	---do----	− − − − − −	---do----	Do.
				76951	----------	---do----	− − − − − −	---do----	Do.
				76952	Nov. 2	----------	(c)	No Bact. abortus lesions	Not cultured.
468	11.8	+ + + + P P	+ + P S − −	76938	----------	---do----	− − − − − −	No visible lesions	Negative.
				76939	----------	---do----	− − − − − −	---do----	Positive.
				76940	----------	---do----	− − − − − −	---do----	Negative.

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17	14.6	+ + P P P P	+ + + P S S	76947	----------	Nov. 24	- - - - - -	No visible lesions	Negative.
				76948	----------	...do....	- - - - - -	.....do.....	Do.
				76949	----------	...do....	- - - - - -	.....do.....	Positive.
71	13.2	+ + + + + +	+ + + P P S	76956	----------	...do....	+ S - - - -	Liver and spleen lesions	Do.
				76957	----------	...do....	+ + P P - -	.....do.....	Do.
				76958	----------	...do....	+ + P P - -	Spleen slightly enlarged.[a]	Do.
84	16.3	+ + + P P S	+ P P S - -	76965	----------	...do....	- - - - - -	No visible lesions	Negative.
				76966	----------	...do....	- - - - - -	.....do.....	Do.
				76967	----------	...do....	- - - - - -	.....do.....	Do.
223	43.4	+ + + + + +	P P P P - -	76944	----------	...do....	+ + + P - -	Spleen and liver lesions	Positive.
				76945	----------	...do....	+ P P - - -	No visible lesions	Do.
				76946	----------	...do....	+ + + + P -	.....do.....	Do.
254	Dry.	+ + + + + +	+ + + + + +	76980	----------	...do....	P S - - - -	.....do.....	Do.
				76981	----------	...do....	+ + + P - -	.....do.....	Do.
				76982	----------	...do....	+ P P - - -	.....do.....	Do.
441	11.5	+ + + + + P	+ P P P - -	76974	Oct. 11	----------	(c)	No autopsy	Not cultured.
				76975	Oct. 12	----------	(b)	.....do.....	Do.
				76976	Nov. 4	----------	(c)	No Bact. abortus lesions	Negative.
465	10.5	P P P P P S	+ P S - - -	76941	----------	Nov. 24	- - - - - -	No visible lesions	Do.
				76942	----------	...do....	- - - - - -	.....do.....	Positive.
				76943	----------	...do....	+ + P - - -	Liver and spleen lesions	Do.
466	Dry.	P P P P P S	+ + P P P P	76983	----------	...do....	+ + + + + P	.....do.....	Do.
				76984	----------	...do....	+ + + + + +	.....do.....	Do.
				76985	----------	...do....	+ + + + + +	.....do.....	Do.

[a] For the 24 hours (Oct. 9, 1922) just prior to taking samples.
[b] All inoculated (Oct. 10, 1922) 5 c. c. milk per pig.
[c] No test.

TABLE VI.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 167 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds[a]	Agglutination results		Guinea-pig inoculations[b]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea pig No.	Date of death (1922)				
					Died	Killed			
247	21.8	+ + + + + +	+ + + P – –	77431	----------	Jan. 29	+ + + P – –	Spleen and liver lesions	Positive.
				77432	----------	...do	+ + + P – –	Testicle lesions	Do.
				77433	----------	...do	+ + + + – –	Spleen nodular	Do.
432	7	+ + + + + +	P P + + S S	77437	Dec. 15	----------	(c)	No B. abortus lesions	Not cultured.
				77438	Dec. 31	----------	(c)	...do	Negative.
				77439	----------	Jan. 29	– – – – – –	...do	Do.
435	21.1	+ + + + S S	+ P – – – –	77440	----------	...do	– – – – – –	No visible lesions	Do.
				77441	----------	...do	+ + + + – –	Spleen enlarged	Positive.
				77442	----------	...do	+ + + + – –	...do	Do.
439	16.8	+ + + P P –	+ + P – – –	77443	----------	...do	– – – – – –	No visible lesions	Negative.
				77444	----------	...do	– – – – – –	...do	Do.
				77445	----------	...do	– – – – – –	...do	Do.
409	14.5	+ + + + + P	+ + P S – –	77446	----------	...do	– – – – – –	...do	Do.
				77447	----------	...do	+ + + + P P	Spleen and liver lesions	Positive.
				77448	----------	...do	– – – – – –	No visible lesions	Negative
442	8.9	+ + + + + +	+ + + P – –	77434	----------	...do	– – – – – –	...do	Do.
				77435	----------	...do	– – – – – –	...do	Do.
				77436	----------	...do	– – – – – –	...do	Do.
468	14.3	+ + + + S –	S S S – – –	77449	----------	...do	+ + + + – –	Spleen much enlarged	Positive.
				77450	----------	...do	+ + + + + P	...do	Do.
				77451	----------	...do	+ + + + S –	...do	Do.

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17	15	+ + + + + −	+ + + P − −	77452	.....	Jan. 29	− − − − − −	No visible lesions	Negative.
				77453	.....	...do.....	− − − − − −	.....do.....	Do.
				77454	.....	...do.....	− − − − − −	.....do.....	Do.
71	10.4	+ + + + + P	+ + + P − −	77455	.....	...do.....	− − − − − −	.....do.....	Do.
				77456	.....	...do.....	− − − − − −	.....do.....	Do.
				77457	.....	...do.....	− − − − − −	.....do.....	Do.
84	17.8	+ + P P P −	P S − − − −	77458	.....	...do.....	− − − − − −	.....do.....	Do.
				77459	.....	...do.....	− − − − − −	.....do.....	Do.
				77460	.....	...do.....	− − − − − −	.....do.....	Do.
223	40.1	+ + + + + +	+ + S − − −	77461	.....	...do.....	+ + + + − −	.....do.....	Positive.
				77462	.....	...do.....	P P − − − −	.....do.....	Negative.
				77463	.....	...do.....	+ + + P − −	.....do.....	Positive.
254	Dry.	+ + + + + P	+ + + + + +	77464	.....	...do.....	+ + P − − −	Spleen and testicle lesions	Do.
				77465	.....	...do.....	+ + + + − −	Spleen nodular	Do.
				77466	.....	...do.....	+ P P P − −	Spleen and testicle lesions	Do.
441	16	+ + + P P −	S − − − − −	77467	.....	...do.....	− − − − − −	No visible lesions	Negative.
				77468	.....	...do.....	− − − − − −	.....do.....	Do.
				77469	.....	...do.....	− − − − − −	.....do.....	Do.
465	Dry.	+ + + + P −	+ + + + + −	77470	.....	...do.....	+ + + S − −	Spleen much enlarged	Positive.
				77471	.....	...do.....	+ + + + + −	Spleen and testicle lesions	Do.
				77472	.....	...do.....	+ + + + + + −	Spleen much enlarged	Do.
466	34.2	+ + + + P −	P P − − − −	77473	.....	...do.....	+ + S − − −	Spleen enlarged	Do.
				77474	.....	...do.....	+ + + + + +	Spleen and liver lesions	Do.
				77475	.....	...do.....	+ + + + + +	Spleen much enlarged	Do.

[a] For the 24 hours (Dec. 5, 1922) just prior to taking samples.
[b] All inoculated Dec. 6, 1922, 5 c. c. milk per pig.
[c] No test.

TABLE VII.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Blood and milk samples collected 312 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds[a]	Agglutination results		Guinea-pig inoculations[b]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
		Blood	Milk	Guinea-pig No.	Date of death (1923)				
					Died	Killed			
247	18	+ + + + + +	+ + + + – –	79980	----------	June 25	+ P S – – –	No visible lesions	Positive.
				79981	----------	---do----	+ + + + + +	---do----	Do.
				79982	----------	---do----	P S – – – –	---do----	Do.
432	21	+ + + + P P	+ P P P – –	79983	----------	---do----	P P P P – –	Spleen enlarged	Do.
				79992	----------	---do----	– – – – – –	No Bact. abortus lesions	Negative.
				79993	May 26	----------	(c)	---do----	Do.
				79994	----------	June 25	– – – – –	---do----	Do.
435	15.9	+ P S S S –	+ + P – – –	79995	----------	---do----	– – – – –	---do----	Do.
				79996	----------	---do----	– – – – –	No visible lesions	Do.
				79997	----------	---do----	– – – – –	---do----	Do.
				79998	----------	---do----	– – – – –	---do----	Do.
439	11.2	P P P + + +	P + P P – –	79999	----------	---do----	– – – – –	---do----	Do.
				80100	----------	---do----	P S – – – –	---do----	Do.
				80101	----------	---do----	– – – – –	---do----	Do.
				80102	----------	---do----	– – – – –	---do----	Do.
409	37.3	+ + + + S –	+ + P P – –	80103	----------	---do----	+ + + + P –	---do----	Positive.
				79988	----------	---do----	– – – – –	---do----	Negative.
				79989	----------	---do----	– – – – –	---do----	Do.
				79990	----------	---do----	– – – – –	---do----	Do.
442	33	P + + + + +	P P + P – –	79991	----------	---do----	– – – – –	---do----	Positive.
				80108	May 26	----------	(c)	No Bact. abortus lesions	Not cultured.
				80109	----------	June 25	– – – – –	---do----	Negative.
				80110	----------	---do----	– – – – –	---do----	Positive.
468	9.9	+ + + + P P	P P P – – –	80111	----------	---do----	– – – – –	---do----	Do.
				80120	----------	---do----	– – – – –	No visible lesion	Do.
				80121	----------	---do----	– – – – –	---do----	Negative.
				80122	May 13	----------	(c)	No Bact. abortus lesions	Not cultured.
				80123	----------	June 25	P P S – – –	---do----	Positive.

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17	9.6	+ + S S S −	+ + P S S −	79964	.....	June 25	− − − − − −	No visible lesions	Negative.
				79965	.....	do	− − − − − −	do	Do.
				79966	.....	do	− − − − − −	do	Do.
				79967	.....	do	− − − − − −	do	Do.
71	Dry.	+ + + + + +	+ + + + + +	79968	May 30	.....	(c)	No autopsy	Not cultured.
				79969	.....	June 25	+ + + + + +	Spleen and liver lesions	Positive.
				79970	.....	do	+ + + + + +	Spleen enlarged	Do.
				79971	.....	do	+ + + + + −	No visible lesions	Do.
84	10.6	+ + + P S −	+ P P P − −	79972	.....	do	− − − − − −	do	Negative.
				79973	.....	do	− − − − − −	do	Do.
				79974	.....	do	− − − − − −	do	Do.
				79975	.....	do	− − − − − −	do	Do.
223	17.9	+ + + + + +	+ + + S − −	79976	.....	do	− − − − − −	do	Do.
				79977	.....	do	+ + + − − −	Spleen slightly enlarged	Positive.
				79978	.....	do	+ + + + − −	No visible lesions	Do.
				79979	.....	do	− − − − − −	do	Negative.
254	51.5	+ + + + + −	+ + S S − −	79984	.....	do	P S S − − −	do	Positive.
				79985	.....	do	− − − − − −	do	Negative.
				79986	.....	do	− − − − − −	do	Do.
				79987	.....	do	+ + + + − −	Spleen nodular	Positive.
441	14.3	+ + P P S S	+ P S S − −	80104	.....	do	− − − − − −	No visible lesions	Negative.
				80105	.....	do	− − − − − −	do	Do.
				80106	.....	do	− − − − − −	do	Do.
				80107	.....	do	− − − − − −	do	Do.
465	24.2	+ + + + + +	P P P − − −	80112	.....	do	+ + P − − −	do	Do.
				80113	.....	do	− − − − − −	do	Do.
				80114	.....	do	− − − − − −	do	Do.
				80115	.....	do	− − − − − −	do	Positive.
466	19.9	+ + P P P P	+ + P S − −	80116	May 31	.....	(c)	No Bact. abortus lesions	Not cultured.
				80117	.....	June 25	+ + P P − −	Liver lesions	Positive.
				80118	.....	do	+ + + + + +	Spleen enlarged	Do.
				80119	.....	do	+ + + + + +	Spleen and liver lesions	Do.

[a] For the 24 hours (May 8, 1923) just prior to taking samples.
[b] All inoculated May 9, 1923, 5 c. c. milk per pig.
[c] No test.

TABLE VIII.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Milk samples collected 458 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield, in pounds [a]	Agglutination results, milk	Guinea-pig inoculations [b]				Autopsy findings in guinea pigs	B. abortus cultural results
			Guinea-pig No.	Date of death (1921)		Agglutination results, guinea-pig blood		
				Died	Killed			
247	19.8	+ + + P S −	81683	Oct. 5		(c)	No Bact. abortus lesions	Not cultured.
			81684		Nov. 7	+ + + S − −	No visible lesions	Positive.
			81685		do	+ + + P S −	do	Do.
432	8.2	+ + P P − −	81689		do	− − − − − −	do	Negative.
			81690		do	− − − − − −	do	Do.
			81691		do	− − − − − −	do	Do.
435	20	+ + S − − −	81692		do	− − − − − −	do	Do.
			81693		do	− − − − − −	do	Do.
			81694		do	+ S − − − −	do	Positive.
439	2.2	+ + + + + +	81695		do	− − − − − −	do	Negative.
			81696		do	− − − − − −	do	Do.
			81697		do	− − − − − −	do	Do.
409	24.6	+ + S S − −	81698		do	− − − − − −	do	Do.
			81699		do	− − − − − −	do	Do.
			81900		do	− − − − − −	do	Do.
442	28.1	+ + P S − −	81686		do	− − − − − −	do	Do.
			81687		do	− − − − − −	do	Do.
			81688	Oct. 27	do	(c)	No Bact. abortus lesions	Not cultured.
468	3.6	+ + + P S −	81901		Nov. 7	+ + S − − −	No visible lesions	Positive.
			81902		do	− − − − − −	do	Negative.
			81903		do	− − − − − −	do	Positive.

CONTROLS

17	Dry.	+ + + P S −	81904	.....	Nov. 7	− − − − − −	No visible lesions	Negative.
			81905	.....	do	− − − − − −	do	Do.
			81906	.....	do	− − − − − −	do	Do.
71	24.8	+ + + P P P	81907	.....	do	+ + + + − −	Spleen nodular	Positive.
			81908	.....	do	+ P P S − −	No visible lesions	Do.
			81909	.....	do	+ + P S − −	Spleen nodular	Do.
84	1	P P P P S −	81910	.....	do	− − − − − −	No visible lesions	Negative.
			81911	.....	do	− − − − − −	do	Do.
			81912	.....	do	− − − − − −	do	Do.
223	10	P P S − − −	81913	.....	do	− − − − − −	do	Do.
			81914	.....	do	P S − − − −	do	Positive.
			81915	.....	do	− − − − − −	do	Negative.
254	34.8	+ + P P − −	81916	.....	do	+ + + P S −	Spleen and liver lesions	Positive.
			81917	.....	do	+ + + P − −	No visibe lesions	Do.
			81918	.....	do	− − − − − −	do	Negative.
441	Dry.	+ + + + + +	81919	.....	do	− − − − − −	do	Do.
			81920	.....	do	− − − − − −	do	Do.
			81921	.....	do	− − − − − −	do	Do.
465	14.4	+ + P − − −	81922	.....	do	− − − − − −	do	Do.
			81923	.....	do	− − − − − −	do	Do.
			81924	.....	do	+ S − − − −	do	Positive.
466	Dry.	+ + P P S −	81925	.....	do	+ + + P P P	Spleen nodular	Do.
			81926	.....	do	+ + S S − −	No visible lesions	Do.
			81927	.....	do	+ + P P S S	Spleen nodular	Do.

[a] For the 24 hours (Sept. 26, 1923) just prior to taking samples.
[b] All inoculated Sept. 27, 1923, 5 c. c. milk per pig.
[c] No test.

TABLE IX.—*Milk yield, agglutination reactions, results of guinea-pig inoculations, etc. Milk samples collected 608 days after bacterin treatment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Milk yield in pounds [a]	Agglutination results, milk	Guinea-pig inoculations [b]			Agglutination results, guinea-pig blood	Autopsy findings in guinea pigs	B. abortus cultural results
			Guinea pig No.	Date of death (1924)				
				Died	Killed			
247	16.1	+ + + + − −	83703	.....	Mar. 29	+ + + + P S	No visible lesions	Positive.
			83704	.....	do	− − − − − −	do	Negative
			83705	.....	do	− − − − − −	do	Do
			83706	.....	do	− − − − − −	do	Do.
432	Dry.	+ + P P P P	83466	Mar. 7	.....	(c)	No Bact. abortus lesions	Do.
			83467	.....	Mar. 29	− − − − − −	No visible lesions	Do.
			83468	.....	do	− − − − − −	do	Do.
			83469	.....	do	− − − − − −	do	Do.
435	10.6	P P P − − −	83497	.....	do	− − − − − −	do	Do.
			83498	.....	do	+ P P S S −	Spleen and liver lesions	Positive.
			83499	.....	do	− − − − − −	No visible lesions	Negative.
			83410	.....	do	− − − − − −	do	Do.
439	40.6	+ P P P − −	83480	.....	do	S − − − − −	do	Positive.
			83481	Mar 26	.....	(c)	No Bact. abortus lesions	Not cultured
			83482	.....	Mar. 29	+ + P P − −	No visible lesions	Positive.
			83483	.....	do	P P S S − −	do	Do.
409	15	P P P P − −	83493	.....	do	− − − − − −	do	Negative.
			83494	.....	do	− − − − − −	do	Do.
			83495	Mar. 17	.....	(c)	No autopsy	Not cultured
			83496	Mar 26	.....	(c)	do	Do.
442	.....	+ + + P P P	83425	Mar. 9	.....	(c)	No Bact. abortus lesions	Do.
			83426	.....	Mar. 29	− − − − − −	No visible lesions	Negative.
			83427	.....	do	− − − − − −	do	Do.
468	42.6	P P S S − −	83470	.....	do	− − − − − −	No visible lesions	Do.
			83471	Mar. 4	.....	(c)	No Bact. abortus lesions	Not cultured.
			83472	Mar. 19	.....	(c)	do	Do.
			83473	.....	Mar. 29	− − − − − −	No visible lesions	Negative.

CONTROLS

17	18	P S S – – –	83485	.....	Mar. 29	– – – – – –	No visible lesions	Negative.
			83486	Mar. 10	.....	(a)	No autopsy	Not cultured.
			83487	.....	Mar. 29	P P P P – –	No visible lesions	Positive.
			83488	.....	do	– – – – – –	do	Negative.
71	7.3	+ + + P P P	83474	.....	do	+ + + S S –	Spleen and liver lesions	Positive.
			83475	.....	do	+ + + + P P	No visible lesions	Do.
			83476	.....	do	+ + + + P P	Spleen enlarged	Do.
			83477	.....	do	+ P P S – –	No visible lesions	Do.
84	32.8	+ S S – – –	83478	Mar. 29	.....	(a)	No Bact. abortus lesions	Negative.
			83479	.....	Mar. 29	– – – – – –	No visible lesions	Do.
			83480	.....	do	– – – – – –	do	Do.
			83481	.....	do	– – – – – –	do	Do.
223	Dry.	P + + + + +	83711	.....	do	P P P P – –	do	Positive.
			83712	.....	do	S – – – – –	do	Do.
			83713	.....	do	– – – – – –	do	Negative.
			83714	.....	do	+ P S S S –	do	Positive.
254	Dry.	P P + + + +	83489	.....	do	+ P S S – –	do	Do.
			83490	.....	do	P P S S S –	Spleen enlarged	Do.
			83491	.....	do	+ P P P P S	Spleen and liver lesions	Do.
			83492	.....	do	+ + + P P P	Spleen enlarged	Do.
441	27.9	+ P – – – –	83707	Mar. 27	.....	(a)	Enteritis	Not cultured.
			83708	.....	Mar. 29	– – – – – –	No visible lesions	Negative.
			83709	.....	do	– – – – – –	do	Do.
			83710	Mar. 16	.....	(a)	No Bact. abortus lesions	Do.
465	7.8	+ + + S – –	83715	.....	Mar. 29	P S S – – –	Spleen and liver lesions	Positive.
			83716	.....	do	+ + + S – –	do	Do.
			83717	.....	do	+ + + P P S	do	Do.
			83718	.....	do	+ + + S – –	No visible lesions	Do.
466	26	P P P P – –	83719	.....	do	+ + P P P P	Spleen enlarged	Do.
			83720	.....	do	+ + + S S –	Spleen and liver lesions	Do.
			83721	.....	do	+ + P P P P	do	Do.
			83722	.....	do	P P S – – –	No visible lesions	Do.

[a] For the 24 hours (Feb. 19, 1924) just prior to taking samples.
[b] All inoculated Feb. 20, 1924, 5 c. c. milk per pig.
[c] No test.

TABLE X.—*Number of guinea pigs which yielded positive or negative cultural results for Bacterium abortus following inoculations with milk from experimental cows in Tables II, IV, V, VI, VII, VIII, and IX*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.[a]	First group (inoculated Mar. 29, 1922)		Second group (inoculated Aug. 24, 1922)		Third group (inoculated Oct. 10, 1922)		Fourth group (inoculated Dec. 6, 1922)		Fifth group (inoculated May 9, 1923)		Sixth group (inoculated Sept. 27, 1923)		Seventh group (inoculated Feb. 20, 1924)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
51	2			3		3								
247	2		2	1	2		3		4		2		1	3
432	1	1	2	1		2		2		4		2		3
435	2	2	2	1		2	2	1		4	1	2	1	3
439	1	1	1	2	1	2		3	1	3		3	3	
409	1	2	1	2	2	1	1	2	1	3		3		2
442	2		2	1		2		3	2	1		2		2
468	2		2	1	1	2	3		2	1	2	1		2
CONTROLS														
17	2			3	1	2		3		4		3	1	2
71	1	1	3		3			3	3		3		4	
84	1	1		3		3		3		4		3		4
223	2	1	1	2	3		2	1	2	2	1	2	3	1
254	1	1	3		3		3		2	2	2	1	4	
441	2			3		1		3		4		3		3
465	3		1	1	2	1	3		1	3	1	2	4	
466	1	1	3		3		3		3		3		4	

[a] Cow No. 51 died after third test.

TABLE XI.—*Agglutination reactions of blood obtained on different dates during the experiments*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Tested Mar. 28, 1922	Tested Aug. 23, 1922	Tested Oct. 9, 1922	Tested Dec. 5, 1922	Tested May 8, 1923
51	+ + + P S S	+ + + + S −	+ + + + + P		
247	+ + + + + −	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +
432	+ + + + + +	+ + + + + +	P P P + + +	+ + + + + +	+ + + + P P
435	+ + + + P S	S S S S P P	+ P P P P P	+ + + + S S	+ P S S S −
439	+ + + + + +	P P + + + +	P P + + + +	+ + + P P −	P P P + + +
409	+ + + + + +	P P P + + +	+ + + + + +	+ + + + + P	+ + + + S −
442	+ + + + + +	P P P P + +	+ P P P + P	+ + + + + +	P + + + + +
468	S S S S S −	P P P + + S	+ + + + P P	+ + + + S −	+ + + + P P
CONTROLS					
17	+ + + P P P	P P S S S S	+ + P P P P	+ + + + + −	+ + S S S −
71	+ + + + P P	P P + + + +	+ + + + + +	+ + + + + P	+ + + + + +
84	+ + + + + S	+ + P P P P	+ + + P P S	+ + P P P −	+ + + P S −
223	+ + + + + +	P + + + + +	+ + + + + −	+ + + + + +	+ + + + + +
254	+ + + + + +	+ + + + P P	+ + + + + +	+ + + + + P	+ + + + + −
441	+ + + + + −	+ + + + P P	+ + + + + P	+ + + P P −	+ + P P S S
465	P P P P P P	P + + + P −	P P P P P S	+ + + + P −	+ + + + + +
466	P P P P P −	+ P P P P P	P P P P P S	+ + + + P −	+ + P P P P

TABLE XII.—*Agglutination reactions of milk obtained on different dates during the experiment*

COWS TREATED WITH BACTERIUM ABORTUS BACTERIN

Cow No.	Tested Mar. 28, 1922		Tested Aug. 23, 1922		Tested Oct. 9, 1922		Tested Dec. 5, 1922		Tested May 8, 1923		Tested Sept. 26, 1923		Tested Feb. 19, 1924	
	Milk yield, pounds *	Results	Milk yield, pounds *	Results	Milk yield, pounds *	Results	Milk yield, pounds *	Results	Milk yield, pounds *	Results	Milk yield, pounds *	Results	Milk yield, pounds *	Results
51	47	+ P P S – –	18.2	+ + P S – –	21.6	P P P P S –								
247	49.3	+ + P S – –		+ + P S – –	18.5	+ P P P – –	21.8	+ + + P – –	18	+ + + + – –	19.8	+ + + P S –	16.1	+ + + + – –
432	24.4	+ + + + + P P	18.6	+ + P P – –	15.4	P P P P P P	7	P P + + S S	21	+ P P P – –	8.2	+ + P P – –	Dry.	+ + P P P P
435	34.6	+ + + P S –	27.5	+ S S – – –	26	+ P P – – – –	21.1	+ P – – – –	15.9	+ + P – – –	20	+ + S – – –	10.6	P P P – – –
439	17.7	+ + + P S –	14	+ + + + P S	20.1	+ + P P – –	16.8	+ + P – – –	11.2	P + P P – –	2.2	+ + + + + +	40.6	+ P P P – –
409	34.4	+ P P P S –	25	+ + + + – –	20.7	+ + + – – –	14.5	+ + P S – –	37.3	+ + P P – –	24.6	+ + S S – –	15	P P P P – –
442	34	+ + + P S –	17.4	+ P P S – –	15.1	+ + P S S –	8.9	+ + + P – –	33	P P + P – –	28.1	+ + P S – –		+ + + P P P
468	23.1	P S S S – –	17.2	+ + P S – –	11.8	+ + P S – –	14.3	S S S – – –	9.9	P P P – – –	3.6	+ + + P S –	42.6	P P S S – –
CONTROLS														
17	9.8	P P P P P S –	18.3	+ P P P P S –	14.6	+ + + P S S	15	+ + + P – –	9.6	+ + P S S –	Dry.	+ + + P S –	18	P S S – – –
71	42.7	+ + P S – –	18.3	+ P P P S –	13.2	+ + + P P S	10.4	+ + + P – –	Dry.	+ + + + + +	24.8	+ + + P P P	7.3	+ + + P P P
84	Dry.	P P P P P P	24	+ + P S – –	16.3	+ P P S – –	17.8	P S – – – –	10.6	+ P P P – –	1	P P P P S –	32.8	+ S S – – –
223	Dry.	+ + + P P S	51	+ + + + S –	43.4	P P P P – –	40.1	+ + S – – –	17.9	+ + + S – –	10	P P S – – –	Dry.	P + + + + +
254	44.5	+ + + + S –	19.6	+ + + + P –	Dry.	+ + + + + +	Dry.	+ + + + + +	51.5	+ + S S – –	34.8	+ + P P – –	Dry.	P P + + + +
441	28.6	+ + + P S –	19.5	P P P – – –	11.5	+ P P P – –	16	S – – – – –	14.3	+ P S S – –	Dry.	+ + + + + +	27.9	+ P – – – –
465	21.8	+ + + + S –	19.6	+ + S – – –	10.5	+ P S – – –	Dry.	+ + + + + –	24.2	P P P – – –	14.4	+ + P – – –	7.8	+ + + S – –
466	24.7	P P P S – –	Dry.	+ + + + + +	Dry.	+ + P P P P	34.2	P P – – – –	19.9	+ + P S – –	Dry.	+ + P P S –	26	P P P P – –

* The milk yield is for the 24-hour period just prior to taking samples.

TABLE XIII.—*Breeding data on experimental cows during period of experiment*

Cow No.	Data
	COWS TREATED WITH BACTERIUM ABORTUS BACTERIN
247	Nonpregnant following 12 services.
409	Calved May 1, 1923 (282 days); pregnant; due to calve May 19, 1924.
432	Calved Feb. 28, 1923 (278 days); pregnant; due to calve Apr. 29, 1924.
435	Calved July 6, 1923 (272 days); pregnant; due to calve July 6, 1924.
439	Aborted Sept. 20, 1923 (178 days); calved Dec. 13, 1924 (278 days).
442	Calved Apr. 30, 1923 (272 days); pregnant; due to calve June 14, 1924.
468	Served 10 times; calved Jan. 5, 1924 (276 days).
	CONTROLS
17	Calved June 15, 1922 (calf vigorous); calved Jan. 8, 1924 (265 days).
71	Calved July 17, 1923 (278 days); served Feb. 27, 1924 (probably pregnant).
84	Aborted July 29, 1922 (243 days); aborted Dec. 31, 1923 (252 days).
223	Calved June 19, 1922 (calf vigorous); calved Mar. 9, 1924 (286 days).
254	Calved Jan. 28, 1923 (281 days); pregnant; due to calve May 7, 1924.
441	Calved Oct. 29, 1923 (280 days); served Jan. 23, 1924 (probably pregnant).
465	Calved Jan. 20, 1923 (277 days); served 10 times; last service Jan. 22, 1924.
466	Calved Oct. 21, 1922 (282 days); calved Sept. 28, 1923 (278 days).

The results from the guinea-pig inoculations failed to indicate that the infective properties of the milk of different cows was influenced greatly by the quantity produced. It was also noted that unless the cows were no longer being milked daily, the quantity of milk produced seemed to have no marked influence on the agglutinin titer of the whey. It was observed, however, that the milk of certain cows, whether they were practically dry or producing from 35 to 40 pounds daily, infected guninea pigs with marked regularity when injected in 5 c. c. quantities. Cows 71 and 466 illustrate this point. The milk of other subjects produced less uniform results in this respect. Whether such variations were due to differences in virulence of strains, numbers of organisms contained in the milk samples, or to the resistance of different pigs was not determined.

SUMMARY

Repeated injections of *Bact. abortus* bacterin, in which the organisms had been killed by heat, did not prove to be of practical value in overcoming *Bact. abortus* udder infection in cows.

Eight of a group of fifteen cows with udders infected by *Bact. abortus* produced milk capable of infecting guinea pigs for approximately two years.

Variations in the quantity of milk produced by a *Bact. abortus* udder-infected cow, provided the animal is milked daily, did not markedly affect the agglutination reaction of the milk whey.

The blood serum of cows which have acquired infectious abortion may react strongly to the agglutination test for at least a year after the disappearance of the infection from the udder, as indicated by guinea-pig inoculation tests.

SHAPE AND WEIGHT OF EGGS IN RELATION TO THEIR HATCHING QUALITY [1]

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INTRODUCTION

During recent years the annual replenishment of the chicken flocks of the country by means of artificial incubation has been accompanied by excessive embryonic mortality. The problem of the hatchability of hen eggs is therefore an important one, inasmuch as there are a number of factors that may cause the death of a relatively large number of embryos in proportion to the total number of fertile eggs artificially incubated. Concerning the various factors possibly contributing toward this heavy embryonic mortality, the reader is referred to a résumé contributed by Dunn.[2] There is need for much more investigational work concerning the effects of the various processes of incubation on embryonic development and variations in respect to the inherent hatching quality of eggs.

Concerning the inherent qualities that possibly affect hatchability Dunn has investigated the relationship between the weight and the hatching quality of White Leghorn eggs. Among other things, he found that for both pullets and hens, the largest eggs did not hatch as well as eggs near the average weight for the flock, and that there was no correlation between the mean weight of the eggs laid by an individual pullet or hen and the proportion of her fertile eggs which hatched. If the first result obtained by Dunn applies to other breeds and varieties of chickens as well as to other strains of White Leghorns, then it becomes important to determine to what extent large eggs should be eliminated from the eggs saved for incubation. On the other hand, the second result noted by Dunn implies that the elimination of the eggs laid by individual birds whose mean egg weight is above the mean for the flock will not affect the proportion of fertile eggs hatching. This apparent paradoxical situation is explained by the fact that Dunn also found that among the eggs of an individual fowl those which were above the mean weight for the individual did not hatch as well as those which were below the mean weight, regardless of the absolute mean egg weight.

In order to ascertain the relationship between weight and hatching quality of eggs in Barred Plymouth Rocks and Rhode Island Reds, the writers have gone over the incubation records of a large number of birds, including pullets and hens, and have prepared the data as presented in the following tables. The relationship between shape

[1] Received for publication January 19, 1920; issued December, 1925.

[2] DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGGS. Conn. Storrs Agr. Exp. Sta. Bul. 109: 93-114. 1922.

and hatching quality of eggs laid by a flock of Barred Plymouth Rock pullets has also been considered, and the data pertaining thereto are presented here.

MATERIAL AND METHODS

The group of birds on which the study of the relationship between egg shape and hatching quality is based consisted of two flocks each of 12 Barred Plymouth Rock pullets mated to Brown Leghorn cockerels. The eggs laid between the first of February and the last of April were dated and carefully measured when laid. The length and maximum breadth of each egg were measured in millimeters, the measurements being recorded to hundredths of a millimeter. The length-breadth index was used as a measure of shape and was obtained by dividing 100 times the breadth by the length. A long and narrow egg has a relatively low index, while a short and broad egg has a high index.

Five groups of birds contributed the material for the study of the relationship between egg weight and hatching quality. The first group consisted of 53 Rhode Island Red hens mated to Rhode Island Red cockerels. The second group consisted of 30 Rhode Island Red pullets mated to Rhode Island Red cockerels. The third group consisted of 50 Barred Plymouth Rock hens mated to Rhode Island Red cockerels. The fourth group consisted of 113 Barred Plymouth Rock pullets mated to Rhode Island Red cockerels. In the case of each of these groups, matings consisted of from 12 to 15 females mated to one male. The fifth group consisted of the two matings of Barred Plymouth Rock pullets with the Brown Leghorn males mentioned. In the case of the first two groups the eggs were laid between March 5 and March 23; the eggs of the second two groups were laid between March 15 and March 23; the eggs of the last group were laid between the first of February and the last of April. The eggs were dated and weighed daily as laid, the weights being recorded to hundredths of a gram.

The eggs of the four largest groups of birds were incubated in a triple-deck mammoth incubator, 5,400-egg capacity, the conditions of the incubation for all the eggs being kept as nearly uniform as possible. The eggs of the fifth group of birds were incubated in an electric incubator of 100-egg capacity, the conditions of incubation also being kept as nearly uniform as possible. All eggs were tested on the seventh day, records being made of the infertile ones and those in which the embryo had died. A second test was made on the fifteenth day, records being made of the embryos which died between the seventh and fifteenth days. On the nineteenth day of incubation the eggs were removed from the incubators, and each egg was placed in a separate hatching sack and replaced in the incubator. On the twenty-second day all hatching sacks were removed from the incubator, and the eggs which hatched were recorded as well as those in which the embryo had died between the fifteenth and the twenty-first days.

In the tables which follow, the data for embryo mortality are divided into two parts, embryos which "died early" and embryos which "died late." The embryos recorded as having died early include all

embryos which died up to and including the fifteenth day of incubation, while those recorded as having died late are the embryos which died after the fifteenth day of incubation.

SHAPE OF EGGS IN RELATION TO HATCHING QUALITY

The evidence on the relationship of egg weight and hatching quality is presented in Tables I, II, and III. In Table I the incubation results of the 1,253 eggs laid by the 24 Barred Plymouth Rock pullets are given, showing for each shape-index class the number of eggs set, the number of infertile eggs, the number which died early, the number which died late, and the number which hatched.

TABLE I.—*Incubation results of eggs for different classes of shape index*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Shape index	Number set	Infertile	Died early	Died late	Hatched
61.00 to 62.99	2				2
63.00 to 64.99	3	1		1	1
65.00 to 66.99	13		2	6	5
67.00 to 68.99	33	1	3	10	19
69.00 to 70.99	89	4	6	21	58
71.00 to 72.99	172	11	14	43	104
73.00 to 74.99	279	28	31	61	159
75.00 to 76.99	295	35	32	54	174
77.00 to 78.99	235	26	23	42	144
79.00 to 80.99	85	7	5	19	54
81.00 to 82.99	35	1	3	15	16
83.00 to 84.99	12	1	2	2	7
Total	1,253	115	121	274	743
Mean shape index	75.07±0.07	75.54±0.18	75.14±0.20	74.89±0.15	75.05±0.08
Standard deviation	3.42± .05	2.92± .13	3.26± .14	3.69± .11	3.40± .06
Coefficient of variability	4.56± .06	3.87± .17	4.34± .19	4.93± .14	4.53± .08

Of the 1,253 eggs set, 1,138 (90.82 per cent) were fertile, and 743 (59.29 per cent) hatched. Of the fertile eggs, 121 (10.63 per cent) died early, 274 (24.08 per cent) died late; and 743 (65.29 per cent) hatched.

The mean shape index for the different groups of eggs was as follows: Number set, 75.07 ± 0.07; infertile, 75.54 ± 0.18; died early, 75.14 ± 0.20; died late, 74.89 ± 0.15; hatched, 75.05 ± 0.08. The differences in mean shape index are very slight and are not significant; as, for instance, the difference with its probable error between the mean shape index of the highest value, 75.54 ± 0.18, and the mean shape index of lowest value, 74.89 ± 0.15, is 0.65 ± 0.23. The relative and absolute amounts of variation in shape index, as shown by the standard deviations and the coefficients of variability, are somewhat similar. Judging from the results as shown in Table I it would seem that the shape of an egg does not affect its hatchability.

The percentage of fertile eggs that hatched for each shape-index class in Table I is not shown, however, since the number of eggs set in some of the shape-index classes was very small and comparisons of hatchability between any two classes would not provide a satisfactory basis of determining the possible influence of shape of egg on hatchability. For instance, in the shape-index class 61.00 to 62.99

2 eggs were set, both of which hatched, giving a hatching percentage of 100, while in the shape-index class 63.00 to 64.99 3 eggs were set, 2 of which were fertile and of which only 1 hatched, giving a hatching percentage of 50 for the fertile eggs. That is, a difference of 1 chick in absolute numbers hatched made a difference of 50 per cent of the fertile eggs that hatched.

In Table II all eggs set have been grouped in classes differing by greater increments of shape index than is shown in Table I, the shape-index classes being divided into three groups, low (61.00 to 68.99), medium (69.00 to 76.99), and high (77.00 to 84.99).

TABLE II.—*Incubation results of eggs for groups of low, medium, and high shape index classes*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Shape index	Number set	Infertile	Died early	Died late	Hatched	Per cent of fertile eggs hatched
61.00 to 68.99	51	2	5	17	27	55.10±7.68
69.00 to 76.99	835	78	83	179	495	65.39± .63
77.00 to 84.99	367	35	33	78	221	66.60±2.75

Of particular interest in Table II is the per cent of fertile eggs hatched in each of the three groups. The low shape-index group shows a hatching percentage of 55.10 ± 7.68, the medium shape-index group 65.39 ± 0.63, the high shape-index group 66.60 ± 2.75. The difference in hatchability between the low and medium groups is 10.29 ± 7.70 and is therefore not significant. The difference in hatchability between the medium and high groups is 1.21 ± 2.82, and also is not significant. The difference in hatchability between the low and high groups is 11.50 ± 8.16, and is not significant. It may be concluded, therefore, that so far as the eggs of the flock as a whole are concerned there is no relationship between the shape of an egg and its hatching quality.

To determine the relation between mean shape index and hatching quality within the individual, the data were regrouped in Table III and show for each pullet the number and mean shape index of the eggs whose embryos died in shell and of the eggs which hatched. The eggs whose embryos died early and those whose embryos died late have been grouped together, because in several cases the numbers of each were too small to be considered separately. Moreover, the factor of importance is to determine the relationship in respect to mean shape index between the eggs which failed to hatch and those which hatched, the infertile eggs not being taken into account.

TABLE III.—*Mean shape index of eggs whose embryos died in shell and that hatched per bird*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Pullet No.	Died in shell		Hatched		Difference
	Number	Mean shape index	Number	Mean shape index	
1	20	76.02±0.53	32	76.55±0.16	0.53±0.55
2	18	75.38± .38	32	75.23± .15	.15± .41
3	17	77.71± .44	42	77.51± .13	.20± .46
4	16	76.87± .27	28	76.80± .17	.07± .32
5	13	78.55± .67	38	78.38± .14	.17± .68
6	13	80.43± .54	33	81.51± .21	1.08± .58
7	12	74.01± .77	34	74.30± .20	.29± .80
8	14	75.93± .37	27	76.98± .27	1.05± .46
9	15	72.48± .62	26	71.97± .14	.51± .64
10	18	77.16± .43	31	75.81± .28	1.35± .51
11	16	73.21± .31	28	73.01± .16	.20± .35
12	13	73.26± .41	35	72.84± .16	.42± .44
13	19	74.03± .22	22	74.53± .24	.50± .33
14	19	76.99± .20	37	76.26± .13	.73± .24
15	22	69.46± .56	28	69.76± .17	.30± .59
16	13	70.43± .41	22	71.22± .21	.79± .49
17	18	77.24± .28	30	76.62± .19	.62± .34
18	18	73.86± .32	29	74.31± .19	.45± .37
19	11	75.38± .61	19	73.83± .24	1.55± .66
20	18	76.31± .21	37	77.00± .15	.69± .26
21	16	77.91± .28	28	77.31± .23	.60± .36
22	14	74.03± .31	34	73.18± .14	.85± .34
23	18	74.37± .24	37	75.02± .16	.65± .29
24	28	78.87± .16	30	78.26± .17	.61± .23

The number of eggs per pullet whose embryos died in shell and those which hatched varied from 30 to 59, with an average of 47.42. The number of eggs whose embryos died in shell varied from 11 to 28 with an average of 16.44 per pullet. The number of eggs which hatched varied from 19 to 42, with an average of 30.98 per pullet.

It is apparent from the data in the last column of Table III that there is no significant difference in any individual pullet between the mean shape index of the eggs whose embryos died in shell and the mean shape index of the eggs which hatched.

The per cent of fertile eggs which hatched has been determined for each of the 24 pullets and the percentages have been correlated with the mean shape index of the fertile eggs of each pullet. The value of this correlation has been found to be 0.27 ± 0.13. Therefore, the mean shape index of a pullet's eggs does not affect their hatchability.

WEIGHT OF EGGS IN RELATION TO HATCHABILITY

In this study, in addition to the 1,253 eggs dealt with previously, the records of 1,392 other eggs are available, including 534 laid by Rhode Island Red hens, 342 laid by Rhode Island Red pullets, 148 laid by Barred Plymouth Rock hens, and 368 laid by Barred Plymouth Rock pullets.

The data pertaining to the second group of eggs are shown in Table IV. For the sake of comparison, the mean weights of the eggs set, those which were infertile, those which died early, those which died late, and those which hatched are shown in comprehensive form. The per cent of fertile eggs which hatched is also shown for each group and for each breed.

TABLE IV.—*Mean weights, classified according to incubation results, of eggs laid by four groups of birds*

[Eggs laid by 53 Rhode Island Red hens and 30 Rhode Island Red pullets]

	Rhode Island Red hens		Rhode Island Red pullets		All Rhode Island Reds	
	Number	Mean weight	Number	Mean weight	Number	Mean weight
		Grams		*Grams*		*Grams*
Set	534	59.82±0.13	342	56.32±0.15	876	58.46±0.11
Infertile	76	60.15± .36	12	55.87± .71	88	59.56± .46
Died early	37	59.83± .43	13	57.65± .50	50	58.78± .37
Died late	100	60.51± .29	65	55.81± .29	165	58.94± .21
Hatched	321	59.64± .17	252	56.36± .17	573	58.20± .14
Per cent of fertile eggs hatched	70.08		76.36		72.71	

[Eggs laid by 50 Barred Plymouth Rock hens and 113 Barred Plymouth Rock pullets]

	Barred Plymouth Rock hens		Barred Plymouth Rock pullets		All Plymouth Rocks	
	Number	Mean weight	Number	Mean weight	Number	Mean weight
		Grams		*Grams*		*Grams*
Set	148	60.25±0.23	368	57.57±0.15	516	58.33±0.13
Infertile	17	59.90± .79	45	57.64± .39	62	58.26± .37
Died early	11	61.50± .66	27	56.76± .42	38	58.13± .41
Died late	8	60.64±1.05	67	57.34± .30	75	57.69± .32
Hatched	112	60.10± .26	229	57.71± .18	341	58.50± .15
Per cent of fertile eggs hatched	85.49		70.90		75.11	

The percentage of fertile eggs that hatched were as follows: Rhode Island Red hens, 70.08; Rhode Island Red pullets, 76.36; all Rhode Island Reds, 72.71; Barred Plymouth Rock hens, 85.49; Barred Plymouth Rock pullets, 70.90; all Barred Plymouth Rocks, 75.11.

It is apparent, from an examination of the mean weights of eggs in each group of birds and in each breed, that there is no significant difference between the mean weights of the eggs whose embryos died either early or late and the mean weights of the eggs that hatched. Apparently, then, in these groups of birds, the relative size of egg has had no effect on hatching quality. Dunn [3] found, however, "an evident tendency for eggs which die during incubation to be slightly larger than those which hatch." Such a situation is not evident in the data shown in Table IV, and in order to test the matter conclusively the more homogeneous lot of 1,253 eggs laid by 24 Barred Plymouth Rock pullets is analyzed.

In Table V are shown the frequency distributions and the mean weights of the eggs set, those which were infertile, those in which the embryos died early, those in which the embryos died late, and those which hatched. In each case the frequency distributions follow fairly closely a normal curve. There are no significant differences between the mean weights of any two groups of eggs. The mean weight in grams is greater in the eggs whose embryos died early, 55.51 ± 0.18,

[3] DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGG. Conn. Storrs Agr. Exp. Sta. Bul. 109, p. 100. 1922.

while among the fertile eggs it is lowest in the eggs which hatched, 55.25 ± 0.10. The difference in weight between these two groups is 0.26 ± 0.21, which is not significant. The greatest variation is among the infertile eggs, while among the fertile eggs the relative and absolute amounts of variation are much the same in the three groups. Here, again, as in the case of the other four groups of birds, there is no evidence of any significant differences among the weights of eggs whose embryos died early, those whose embryos died late, and those which hatched.

TABLE V.—*Incubation results of eggs for different weight classes*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Weight	Number set	Infertile	Died early	Died late	Hatched
Grams					
46.00 to 46.99	2			1	1
47.00 to 47.99	4	1		1	2
48.00 to 48.99	7	2	1	1	3
49.00 to 49.99	11	2	1	3	5
50.00 to 50.99	13	3	3	2	5
51.00 to 51.99	28	4	2	5	17
52.00 to 52.99	77	7	6	14	50
53.00 to 53.99	164	12	14	32	106
54.00 to 54.99	218	19	22	48	129
55.00 to 55.99	227	21	20	52	134
56.00 to 56.99	196	17	16	42	121
57.00 to 57.99	168	11	20	43	94
58.00 to 58.99	81	8	7	16	50
59.00 to 59.99	29	3	4	8	14
60.00 to 60.99	13	2	4	3	4
61.00 to 61.99	6	1	1	1	3
62.00 to 62.99	3	1			2
63.00 to 63.99	2			1	1
64.00 to 64.99	1				1
65.00 to 65.99	2	1			1
66.00 to 66.99	1			1	
Total	1,253	115	121	274	743
Mean weight	55.28±0.04	55.13±0.18	55.51±0.15	55.31±0.10	55.25±0.05
Standard deviation	2.22± .03	2.82± .13	2.37± .10	2.35± .07	2.22± .04
Coefficient of variability	4.20± .06	5.12± .23	4.27± .19	4.25± .12	4.02± .07

The same data as shown in Table V are grouped in Table VI in three classes of weight increments, low (46.00 to 52.99 grams), medium (53.00 to 59.99 grams), and high (60.00 to 66.99 grams).

TABLE VI.—*Incubation results of eggs for groups of low, medium, and high weight classes*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Weight	Number set	Infertile	Died early	Died late	Hatched	Per cent of fertile eggs hatched
Grams						
46.00 to 52.99	142	19	13	27	83	67.48±2.04
53.00 to 59.99	1,083	91	103	241	648	65.32±1.35
60.00 to 66.99	28	5	5	6	12	52.17±9.46

The low-weight group contains a slightly larger percentage of fertile eggs that hatched than the medium-weight group, while the high-weight group contains a percentage of fertile eggs that hatched considerably lower than either of the others. This situation is much the same as that reported by Dunn [4] for White Leghorn pullets, except that his medium-weight group (52.00 to 59.99 grams) contained the largest percentage of fertile eggs that hatched, although the difference in hatchability between his low (eggs weighing up to 51.99 grams) and medium-weight groups was not significant. The difference in percentage of hatchability between the low- and medium-weight groups in this study on Barred Plymouth Rocks is 2.16 ± 2.45; between the medium- and high-weight groups it is 13.15 ± 9.55; between the low- and high-weight groups it is 15.31 ± 9.67. None of these differences in percentage of fertile eggs hatched are significant, whereas Dunn obtained significant differences between his medium- and high-weight groups and between his low- and high-weight groups. Briefly, then, in Dunn's White Leghorn pullets the larger eggs appear to have been somewhat less hatchable than those of small and medium size, while in the case of eggs laid by the Barred Plymouth Rocks used in this study the larger eggs also gave a lower hatching percentage, but no significance is attached to it.

Up to the present, the matter of a possible relationship between egg weight and hatching quality has been considered from the standpoint of the eggs from the flock as a whole. Since the results of several lines of investigation have shown, however, that variation in hatching quality of eggs has been found to be chiefly a variation among individual fowls, it becomes necessary to consider the data shown in Tables V and VI on the basis of the mean weight of each pullet's eggs whose embryos died in shell and those which hatched, in relation to the percentage of fertile eggs which hatched. In other words, is there any correlation between the mean weight of the eggs of individual pullets and the percentage of their fertile eggs which hatched? To show something on this point, the mean weight of the eggs whose embryos died in shell and the mean weight of those which hatched for each pullet are given in Table VII.

[4] DUNN, L. C. THE RELATIONSHIP BETWEEN THE WEIGHT AND THE HATCHING QUALITY OF EGGS. Conn. Storrs Agr. Exp. Sta. Bul. 109: 93-114. 1922.

TABLE VII.—*Mean weight of eggs whose embryos died in shell and that hatched per bird*

[Eggs laid by 24 Barred Plymouth Rock pullets]

Pullet No.	Died in shell		Hatched		Difference
	Number	Mean weight	Number	Mean weight	
		Grams		*Grams*	
1	20	55.28±0.23	32	54.69±0.16	0.59±0.28
2	18	54.64±.18	32	54.91±.12	.27±.22
3	17	54.26±.21	42	54.50±.16	.24±.26
4	16	54.28±.20	28	54.32±.17	.04±.26
5	13	55.25±.13	38	55.32±.14	.09±.19
6	13	55.37±.16	33	55.25±.12	.12±.20
7	12	55.43±.24	34	55.65±.14	.22±.28
8	14	54.44±.21	27	54.31±.18	.13±.28
9	15	58.43±.28	26	57.74±.13	.69±.31
10	18	57.60±.17	31	58.21±.14	.61±.22
11	16	54.45±.16	28	54.24±.16	.21±.23
12	13	56.56±.18	35	56.90±.11	.34±.21
13	19	56.65±.17	22	57.26±.21	.61±.27
14	19	56.68±.15	37	56.51±.10	.17±.18
15	22	57.17±.21	28	57.36±.11	.19±.24
16	13	55.23±.18	22	55.10±.21	.13±.28
17	18	55.52±.14	30	55.34±.14	.18±.20
18	18	54.90±.15	29	55.27±.16	.37±.22
19	11	54.88±.20	19	54.41±.21	.47±.29
20	18	54.89±.14	37	54.96±.13	.07±.19
21	16	55.33±.16	28	55.18±.12	.15±.20
22	14	55.46±.18	34	55.32±.11	.14±.21
23	18	54.34±.16	37	54.85±.12	.51±.20
24	28	55.10±.13	30	54.66±.11	.44±.17

The average number of fertile eggs per pullet was 47.42, with a range of from 30 to 59 eggs. The average number of eggs whose embryos died in shell was 16.44 per pullet, with a range of from 11 to 28 eggs. The average number of eggs which hatched was 30.98 per pullet, with a range of from 19 to 42 eggs.

The mean weight of fertile eggs per pullet varied from 54.30 grams to 57.95 grams, with a mean weight of 55.29 ± 0.05 grams for all pullets. The mean weight of the eggs whose embryos died in shell varied from 54.26 grams to 58.43 grams per pullet, with a mean weight of 55.38 ± 0.08 grams for all pullets. The mean of the mean weights in grams of the eggs whose embryos died in shell was 55.50 ± 0.61 per pullet. The mean weight of the eggs which hatched varied from 54.24 grams to 58.21 grams per pullet, with a mean weight of 55.25 ± 0.05. grams for all pullets. The mean of the mean weights of the eggs which hatched was 55.51 ± 0.64 grams per pullet. Considering the means of the mean weights in grams per pullet of the eggs whose embryos died in shell and those which hatched, it is seen that there is very little difference, 55.50 ± 0.61 and 55.51 ± 0.64. The difference in grams, with its probable error between these two means is 0.01 ± 0.88, which, of course, is not significant. Also, the difference in grams for all pullets between the mean weight of the eggs whose embryos died in shell, 55.38 ± 0.08, and the mean weight of the eggs which hatched, 55.25 ± 0.05, is 0.13 ± 0.09, which is not significant.

The mean weight in grams of the fertile eggs, and the per cent of fertile eggs which hatched per pullet, have been correlated for the group of 24 pullets. The correlation has been found to be 0.25 ± 0.13. There is a slight negative correlation, but it is not significant.

SUMMARY

The results of this study of the shape and weight of eggs in relation to hatching quality, using eggs laid by Barred Plymouth Rock pullets and hens and Rhode Island Red pullets and hens, seem to justify the following conclusions:

Egg shape, where normal eggs are involved, does not affect hatching quality.

There are no significant differences in the mean shapes of eggs whose embryos die up to the fifteenth day, those whose embryos die between the fifteenth and twenty-second days of incubation, and those which hatch.

There is no significant correlation between the mean shape of the eggs laid by an individual bird and the proportion of her fertile eggs which hatch.

The selection of eggs for incubation purposes according to shape can not be expected to affect the hatching results.

Egg weight, where normal eggs are involved, has no bearing on hatching quality.

There are no significant differences in the mean weights of eggs whose embryos die up to the fifteenth day, those whose embryos die between the fifteenth and twenty-second days of incubation, and those which hatch.

There is no significant correlation between the mean weight of the eggs laid by an individual bird and the proportion of her fertile eggs which hatch.

The selection of eggs for incubation purposes according to size can not be expected to affect the hatching results.

RELATION BETWEEN THE BACTERIAL COUNT OF WHOLE MILK AND THAT OF THE CREAM AND SKIM MILK SEPARATED FROM IT [1]

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INTRODUCTION

In studying the milk ordinances of many municipalities it is found that when a maximum bacterial count for milk is designated, a much higher maximum bacterial count for cream of the same grade is allowed. This increase covers a wide range, varying usually from 300 to 500 per cent. A review of the literature upon this subject shows that but little work has been done, or at least few results have been published, dealing with the effect of separation upon the bacterial count of cream and skim milk. It would seem that the maximum bacterial standards for cream appearing in many milk ordinances are not based on scientific facts.

The Bureau of Dairying of the United States Department of Agriculture has been carrying on work with centrifugal and gravity separators in order to determine what effect separation has upon the bacterial count of the cream and skim milk.

CENTRIFUGAL SEPARATION

A steam-turbine separator with a capacity of 1,350 pounds per hour was used. It was run at its rated capacity. After use, the separator parts were cleaned with hot water containing a strong washing powder, then thoroughly rinsed with hot water, and steamed for three minutes over an open steam jet. After cooling, the parts were assembled, again steamed, and then placed in a clean inclosed cabinet until the next run.

After the separator had attained its proper speed, mixed-herd milk at a temperature between 80° and 85° F. was separated. Samples of the whole milk, cream, and skim milk were taken after separation had been in progress about three minutes. The milk sample was taken at the outlet of the supply bowl, and the cream and skim milk samples from their respective spouts. The samples for bacteriological analysis were immediately plated. Standard methods for bacteriological analysis of milk, as recommended by the American Public Health Association,[2] were followed. Plates were incubated for 48 hours at 37.5° C. Fat determinations were made with the Babcock fat test. Samples were taken from 100 separate runs of

[1] Received for publication Jan. 23, 1925; issued December, 1925.

[2] AMERICAN PUBLIC HEALTH ASSOCIATION AND ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. STANDARD METHODS OF MILK ANALYSIS. BACTERIOLOGICAL AND CHEMICAL. Ed. 4, 40 p., illus. New York City. 1923.

the separator. The average bacterial count of 100 samples of whole milk was 435,240 per cubic centimeter. The cream and skim milk resulting from the separation of the milk gave average bacterial counts of 500,830 and 312,740 per cubic centimeter, respectively. The average per cent butterfat of the whole milk was 3.905, and that of the cream 34.64. The per cent fat in the skim milk was 0.02. The average increase of the bacterial count of all the cream samples over the milk from which it was obtained was 14.84 per cent. Twenty-three of the 100 samples of cream showed a lower count than the whole milk. The per cent decrease for these samples was 14.06. Seven samples gave the same count for the cream as for the whole milk. The increase in 70 samples of cream showing an increase over whole milk was 23.87 per cent. (See Table I.)

TABLE I.—*Increase and decrease of bacteria per cubic centimeter in cream as compared with whole milk*

	Per cent
Increase in bacteria per cubic centimeter of 100 samples of cream over the whole milk from which the cream was separated	14. 84
Increase in bacteria per cubic centimeter of the 70 samples of cream showing increase	23. 87
Decrease in bacteria per cubic centimeter of the 23 samples of cream showing decrease	14. 06

Seven samples gave same count as whole milk.

Both high and low count milk was used. The percentage of fat varied from 3.4 per cent to 5.0 per cent. The bulk of the samples gave a bacterial count between 25,000 and 100,000 bacteria per cubic centimeter. (See Tables II and III.)

TABLE II.—*Range and average of bacterial counts and butterfat determinations in 100 samples of whole milk and in the cream and skim milk separated from it by centrifuge*

	Bacteria per cubic centimeter		Percentage of butterfat	
	Range	Average	Range	Average
Whole milk	9,000–14,410,000	435,240	3.4– 5	3.905
Cream	7,000–18,600,000	500,830	17.5–54	36.64
Skim milk	9,000– 7,500,000	312,740	----------	----------

TABLE III.—*Samples grouped according to bacterial counts*

	Bacteria per cubic centimeter						
	10,000 and lower	10,001 to 25,000	25,001 to 50,000	50,001 to 100,000	100,001 to 500,000	500,001 to 1,000,000	1,000,001 and higher
Number of samples:							
Whole milk	4	13	38	20	8	7	9
Cream	2	16	33	22	11	7	9
Skim milk	4	29	27	13	8	4	9

When the same samples are grouped according to their bacterial count, it appears that the greatest per cent of increase in the cream

over the whole milk occurs with milk of low bacterial count. The greatest per cent of decrease also occurs with low-count milk. Part of this variation is possibly due to limits of laboratory error, which would be more pronounced in a low-count than in a high-count milk when figuring percentage increases and decreases.

Table IV gives the grouping of the samples according to bacterial count, together with the percentage relationship of the counts of the cream and whole milk by bacterial groups.

TABLE IV.—*Per cent increase and decrease of bacterial counts of cream and whole milk, classified by grouping of whole milk bacterial count*

Bacteria per cubic centimeter	Increase in cream over whole milk		Decrease in cream under whole milk		Number of samples showing no change
	Number of samples	Average per cent	Number of samples	Average per cent	
0 to 50,000	37	28.07	12	15.907	6
50,001 to 100,000	17	25.2	3	13.13	1
100,001 to 500,000	4	14.82	4	14.722	----
500,001 to 1,000,000	4	10.245	3	9.396	----
1,000,001 and over	8	10.936	1	6.06	----
Total	70	----	23	----	7

Out of a total of 70 samples showing an increase in the bacterial count of the cream over the whole milk, 64 samples (or 91.4 per cent) gave an increase of 50 per cent or less. In no instance was there a greater increase than 90 per cent. All of the 23 cream samples which showed a decrease in the count under that of the original whole milk gave a decreased count of 50 per cent or less.

From the work which has been presented, it would seem that there is no basis of fact for the assumption that the bacterial count of centrifugally separated cream should be several hundred per cent higher than the whole milk from which it is derived, provided the separator is clean.

GRAVITY SEPARATION

For this work a gravity separation can 18 inches high and 8½ inches in diameter was used. At the bottom was placed a pet cock to draw off the skim milk. A small strip of glass was inserted in the side, extending upward from the bottom of the can sufficiently so that the dividing line between the cream and skim milk could be observed. As a means for holding the milk in the separator at a low temparature, the can was placed in a large tank with sufficient ice and water around it to extend above the depth of the milk in the can.

Before the separator can was used it was thoroughly washed with hot water containing a strong solution of washing powder, then thoroughly rinsed, and finally inverted over a steam jet and allowed to steam for five minutes. The cover was cleaned and steamed in the same manner. After steaming, the cover was placed on the can and the separator was allowed to cool. Fresh mixed-herd milk was then placed in the separator. Samples for bacterial count and fat content were taken. The can was then set into the cooling and

storage tank. The samples for bacterial count were suspended in the ice water, thus assuring that storage conditions would be the same for both the whole-milk sample and the separator can. At the end of 24 hours the separator can was taken out and the skim milk drawn off through the pet cock. Samples for bacterial count and fat test were taken. The cream was then drawn off and samples taken, all being 24 hours old. The average temperature at the beginning of the holding time was 38.44° F. At the end of 24 hours, when the skim milk and cream were drawn off, the average temperature was 45.88° F. The highest individual temperature was 52° F.

Bacterial counts and fat determinations were made the same as with the centrifugal separation samples. Twenty-five lots of milk were separated by this method.

In no case was it found that the cream had a lower count than the whole milk. Two out of twenty-five samples of skim milk showed an increase in the bacterial count over that of the whole milk, while 23 samples gave a lower count, The average counts per cubic centimeter of the 25 samples were: Whole milk, 135,880; cream, 283,680; skim milk, 33,556.

The average per cent increase in bacterial count of the cream over the whole milk was 160.86 per cent. The average fat percentages were: Whole milk, 4.208; cream, 20.52; skim milk, 1.22.

The bacterial counts in the milk varied from 2,000 to 850,000 per cubic centimeter. The majority of the samples gave a bacterial count of 100,000 or lower. The average percentage of butterfat was 4.208, but two samples were 5.0 per cent or higher. (See Tables V and VI.)

TABLE V.—*Range and average of bacterial counts and butterfat determinations in 25 samples of whole milk and in the cream and skim milk separated from it by gravity*

	Bacteria per cubic centimeter		Percentage of butterfat	
	Range	Average	Range	Average
25 samples whole milk	2,000– 850,000	135,880	3.0– 5.8	4.208
25 samples cream	5,000–1,700,000	283,680	14.0–24.0	20.52
25 samples skim milk	900– 180,000	33,556	0.5– 2.0	1.22

TABLE VI.—*Samples grouped according to bacterial count*

	Bacteria per cubic centimeter						
	10,000 and under	10,001 to 25,000	25,001 to 50,000	50,001 to 100,000	100,001 to 500,000	500,001 to 1,000,000	1,000,001 and over
Number of samples:							
Whole milk	4	7	4	3	4	3	0
Cream	1	1	7	4	7	2	3
Skim milk	14	4	1	4	2	0	0

In contrast with centrifugally separated cream, gravity separated cream shows a much higher percentage increase in bacterial count over whole milk. The low-count milk gives the greatest increase in bacterial count in the cream. Table 7 shows the groupings of the milk according to bacterial counts, together with percentage increase of cream over whole milk of each group.

TABLE VII.—*Per cent of increase in bacterial count of gravity cream over that of whole milk, classified by groupings of whole-milk bacterial count*

Bacterial count of whole milk	Number of samples	Average per cent of increase	Number of samples showing no change
0 to 50,000	15	198.96	0
50,001 to 100,000	3	156.34	0
100,001 to 500,000	4	89.88	0
500,001 to 1,000,000	3	95.53	0
1,000,001 and over			
Total	25		0

CONCLUSIONS

Centrifugal separation with a clean separator will not result in cream having a greatly higher bacterial count than the whole milk from which the cream is obtained.

Gravity separated cream will give a much higher bacterial count than the whole milk from which it comes.

In view of the fact that gravity cream plays a very minor part in the market-cream trade, it would seem that those milk ordinances allowing cream of a certain grade with a bacterial count hundreds per cent greater than milk of the same grade are based upon custom rather than scientific investigations. High counts in market cream may very probably be due to a poor quality of milk used for separation and to lack of care in sterilizing equipment rather than to any normal causes involved in the process of separation.